Identification of Larval Lake Whitefish (*Coregonus clupeaformis*) and Cisco (*Coregonus artedii*), from Southern Indian Lake, Manitoba by Pigmentation Characteristics and by Isoelectric Focusing of Whole Body Protein Extracts

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IDENTIFICATION OF LARVAL LAKE WHITEFISH (*Coregonus clupeaformis*) AND CISCO (*Coregonus artedii*), FROM SOUTHERN INDIAN LAKE, MANITOBA BY PIGMENTATION CHARACTERISTICS AND BY ISOELECTRIC FOCUSING OF WHOLE BODY PROTEIN EXTRACTS

by

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This is the 200th Technical Report

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ABSTRACT

Fudge, R.J.P., R.A. Bodaly, and M. Viljanen. 1986. Identification of larval lake whitefish (Coregonus clupeaformis) and cisco (Coregonus artedii) from Southern Indian Lake, Manitoba by pigmentation characteristics and by isoelectric focusing of whole body protein extracts. Can. Tech. Rep. Fish. Aquat. Sci. 1471: iv + 30 p.

Problems in identification of larval forms of the genus Coregonus using published keys and morphological examination were encountered during field studies of distribution and abundance. To overcome this problem, cultures of larval lake whitefish (Coregonus clupeaformis) and cisco (Coregonus artedii) were hatched and reared from known parental stock. Morphological and meristic investigations of these cultures, although indicating significant differences between species, proved largely inadequate for species identification and separation. Larval whitefish and cisco could be identified on the basis of pigmentation and mouth position differences. Dorsal pigmentation patterns were useful on fish up to 18 mm standard length (S.L.) while ventral pigmentation was effective on larger specimens. Mouth position was useful to separate fish over 26 mm S.L. Biochemical differences were also determined using isoelectric focusing. These techniques confirmed that identifications of larval coregonids, utilizing keys based on pigmentation, were valid.

Key words: lake whitefish; cisco; melanophores; isoelectric focusing; larval fish.

RESUME

Fudge, R.J.P., R.A. Bodaly, and M. Viljanen. 1986. Identification of larval lake whitefish (Coregonus clupeaformis) and cisco (Coregonus artedii) from Southern Indian Lake, Manitoba by pigmentation characteristics and by isoelectric focusing of whole body protein extracts. Can. Tech. Rep. Fish. Aquat. Sci. 1471: iv + 30 p.

L'identification des sujets du genre Coregonus au stade larvaire à l'aide des clefs établies et par examen morphologique a posé des difficultés au cours d'études de distribution et d'abondance faites sur le terrain. Pour les résoudre, des larves de grand corégone (Coregonus clupeaformis) et de cisco de lac (Coregonus artedii) ont été obtenues; les stocks d'origine étaient connus. Les examens morphologiques et méristiques de ces cultures, bien qu'elles permettent de relever les grandes différences entre les espèces, ne convenaient à peu près pas à l'identification et à la différenciation des espèces. Le corégone et le cisco à l'état larvaire ont pu être identifiés d'après la pigmentation et la position de la bouche, qui sont différentes. La pigmentation dorsale a pu servir à différencier les sujets dont la longueur standard (L.S.) ne dépassait pas 18 mm tandis que la pigmentation ventrale était utilisée pour les sujets plus gros. La position de la bouche a permis de distinguer les sujets de plus de 26 mm (L.S.). Les différences biochimiques ont également été établies au moyen de l'électrofocalisation. Ces techniques ont permis de confirmer la validité de l'identification des larves de coregonidae à partir des clefs établies d'après la pigmentation.

Mots-clés: grand corégone; cisco; mélanophore; électrofocalisation; larve de poisson.

INTRODUCTION

The objectives of this study were to investigate the morphological, meristic and biochemical characteristics of hatchery reared lake whitefish (Coregonus clupeaformis) and cisco (Coregonus artedii) from Southern Indian Lake, Manitoba in order to develop a key for the separation of larval forms.

The subfamily Coregoninae is notorious for extreme morphological plasticity and evolutionary diversity. This has led to difficulties in evaluations based upon standard morphological analyses and prompts investigations of methods other than those of classical taxonomy (Chellevold 1970).

Thus, a multi-faceted approach to studying the problem was initiated. The traditional morphological measurements and meristic counts were performed. In addition, the differences in pigmentation patterns (melanophore distribution) on the dorsal, lateral and ventral surfaces of larval coregonid fish were investigated. Such differences have long been known as potential and effective methods of identification (Pritchard 1930; Fish 1932; Lindström 1962; Faber 1970; Rechkan 1970). Hinrichs (1979) successfully utilized pigmentation to aid in the separation of several coregonid species at different growth stages. Cucin and Faber (1985) applied pigmentation differences between lake whitefish and cisco to aid in identification.

Biochemical studies were also undertaken as a potential means of separating coregonid species. Gel electrophoresis has proven useful for the study of population structure within one coregonid species (Vuorinen et al. 1981) as well as for species identification between morphologically similar salmonids (Vuorinen and Piironen 1984). Recent developments in isoelectric focusing (I.E.F.) have proven it to be an invaluable tool in the differentiation of morphologically similar species (Bishop 1979). Researchers have successfully applied I.E.F. to the specific problem of fish species identification (Lundström 1979, 1980, 1981(a) and (b); Lundstrom and Roderick 1979; Mackie and Ritchie 1981). Djupsund (1976) was able to clearly identify several species of coregonids using electrofocusing. Lundstrom and Roderick (1979) were able to separate 12 species of marine fish by electrofocusing sarcoplasmic proteins. Yamada and Suzuki (1982) separated the majority of 41 fish species, including some which exhibited polymorphic proteins, by studying the species specific gel patterns. We chose to utilize I.E.F. as the method of biochemical research.

This study was initiated to determine a reliable identification procedure for larval coregonids so that field samples, collected for the determination of larval abundance and distribution, could be identified. This integrated approach was felt to give a better overall chance for success.

MATERIALS AND METHODS

FISH REARING

The eggs of lake whitefish and cisco from Areas 4 and 6 on Southern Indian Lake (SIL), Manitoba (Fig. 1) were artificially fertilized in a 3:1 (male to female) ratio utilizing the dry method (Wood and Dunn 1948). Although shortjaw cisco (Coregonus zenithicus) may also be present in SIL (Dr. Redmond Clarke, Freshwater Institute, Winnipeg, personal communication), nothing is known of the percentage composition of this cisco in the lake population nor was an attempt made to clarify the status of adult cisco used in the egg crosses. However, the likelihood of shortjaw being used in crosses was small. The eggs were kept in cages placed in the lake. Periodic cleaning and culling of non-viable eggs kept the cultures healthy. The eggs were surface disinfected using a bridine solution (Amend 1974) and transported 1100 kms to hatchery facilities within the Freshwater Institute, Winnipeg. The eggs were reared in a flow-through aquaria system. Thio-sulphate dechlorinated water was cooled to 3.0°C within a 2200 L head tank. The eggs were placed in round bottom hatchery jars and water flow was adjusted to a level sufficient to keep eggs gently rolling. Malachite green was used on several occasions utilizing the California flush method in an attempt to control fungal growth (Leitritz 1959). It was found that surface fungal growth could be abraded away by gently rolling eggs on a fine mesh screen under sufficient water flow. Hatching occurred after 112 days. It was preceded by a slow temperature rise to 4.5°C over a week and initiated by a sudden rise to 6.5°C. Both species hatched simultaneously and completely within 16 h. The larval fish were reared for six months in aquaria at 10.0°C. They were fed the brine shrimp Artemia (live culture) initially and slowly switched to a diet of finely ground dry fish food (Tetramin (TM) and trout starter).

MORPHOLOGY AND MERISTICS

Samples of larval whitefish and cisco for reference purposes only were removed weekly and preserved directly in 5.0% non-buffered formalin. A further sample was taken and individuals of these fish from each species were studied morphologically and meristically prior to fixation. Total and standard (notochord) lengths were measured by ocular micrometer to the nearest 0.1 mm for a size range extending from time of hatch to 32 mm S.L. (38 mm T.L.). The following morphological measurements were taken with an ocular micrometer to the nearest 0.01 mm: body depth, head depth, head length, eye diameter, pupil diameter and pelvic and pectoral fin lengths (Fig. 2). Meristic counts were done for pre- and post-anal myomeres (muscle plates), dorsal, anal and caudal finrays where possible.

PIGMENTATION AND MOUTH POSITION

Preserved larval lake whitefish and cisco were examined for mouth position and pigmentation data as follows. The incidence of three ventral pigmentation patterns was noted:

pigment scattered on foregut randomly, pigment forming a distinct line on foregut or absence of pigment. The incidence of two dorsal patterns was noted: pigment forming an even double row along entire dorsal surface or pigment concentrated posteriorly. Mouth position was determined as being terminal, inferior or superior. The coded data was plotted against standard length for analysis.

Samples of each species covering the size range from hatching to 32 mm S.L. were photographed using black and white negative film (35 mm). Six views were taken per fish: lateral head, anal fin area (closeup), ventral anterior gut (closeup), lateral, ventral and dorsal whole body. From these photographs a series of drawings was constructed to illustrate pigmentation and mouth position differences at three growth stages; 13 mm, 18 mm and 29 mm S.L. According to Snyder (1976) these stages could be described as follows: 13 mm as late protolarvae/early mesolarvae, 18 mm as metalarvae and 29 mm as juvenile. The drawings are generalized, not drawn to scale and are purposely simplistic so as to accentuate and illustrate the pigmentation and mouth position differences (Faber and Gadd 1983).

ISOELECTRIC FOCUSING (I.E.F.)

Whole larval fish, with gut removed, were macerated in a 0.25 M sucrose extraction solution (with 300 $mg \cdot L^{-1}$ nicotinamide-adenine dinucleotide added) in a 3:1 sucrose solution to fish ratio by weight. After centrifugation at 17,000 r.p.m. for 20 min at 2.0°C, the clear supernatant was pipetted off and frozen. This fraction was used directly for I.E.F.

The polyacrylamide gel utilized was a modification of a formula reported by Valkonen et al. (1980) (Table 1). The gel solution was syringed between two glass plates and photopolymerized for two hours. The resulting gel measured 22.0 x 11.0 x 0.8 mm, was bonded to a slightly larger gelbond mylar sheet and had a pH range of 2.5-11. The gels were kept covered and refrigerated prior to use (maximum 4 days).

The electrolytes (anode - 1.0 M H_3PO_4; cathode - 1.0 M NaOH) were applied to electrofocusing strips and laid directly on the gel surface such that protein migration was along the gel's longitudinal axis, for better band resolution. Prefocusing was carried out for 0.5 h on the bed of an LKB Multiphor 2117, using 2.0 W constant power supplied by a Pharmacia electrophoresis power supply. Exactly 20 µL of protein extract was micropipetted onto pieces of blotting paper (10 x 15 mm) laid on gel surface at any position between cathode and anode (anodal application most commonly used). Focusing proceeded for 18 h at 2.0 W constant power. After the first hour the sample application papers were removed from the gel surface. All electrofocusing was done on apparatus cooled to 2.0°C.

Following focusing, the gel was placed in fixative (57.5 g trichloroacetic acid, 17.25 g sulphosalicylic acid and 500 mL distilled water) for 25 min then washed in destain solution (500 mL ethanol, 160 mL acetic acid and 1340 mL distilled water) for 10 min. The gel was then stained (0.46 g Coomassie brilliant blue R-250 in 400 mL of destain) for 10 min. The final process of destaining required several changes of destaining solution over one day until bands were clearly discriminated. The gels were covered in a thin mylar film and refrigerated in whirlpak bags. The number of bands and banding patterns were visually interpreted from black and white photographs. A few reference pH determinations were made on some gels immediately following focusing, utilizing a Fisher Accumet model 825 meter with an Ingold 6122 surface electrode.

RESULTS

MORPHOLOGY

Morphological measurements on whitefish and cisco larvae are summarized within four length intervals (Table 2). Within each length class, mean morphological measurements were generally larger for whitefish. Analysis of covariance showed statistically significant differences between whitefish and cisco for six characters (Table 3). Where this difference occurred between the adjusted group means (body depth, head length, eye diameter, pupil diameter, pelvic fin length and pectoral fin length), values were larger for whitefish than cisco, except for body depth. For characters which differed in slope of the regression lines (head length, eye diameter, pelvic fin length and pupil diameter), those for whitefish were steeper than those for cisco. Although these differences were significant, plots of the various morphological characters versus standard length for both species show large overlapping distributions (Figs. 3-9).

MERISTICS

The equality of least squares mean meristic counts for lake whitefish and cisco was tested using analysis of variance (Table 4). The mean dorsal fin ray counts were not significantly different and the modal values were identical (Fig. 10). The mean anal fin ray counts were not significantly different (Table 4). The modal anal fin ray count was higher for cisco but the overall distribution of values was larger in whitefish (Fig. 11). The mean caudal fin ray counts were not significantly different (Table 4). Caudal fin ray count distributions appeared bi-modal with values in both modes higher for cisco than whitefish (Fig. 12). The mean post-anal myomere counts were not significantly different (Table 4). The post-anal myomere count distributions were similar with modal values identical for both species (Fig. 13). The mean pre-anal myomere count was significantly greater for whitefish (Table 4). The distribution of pre-anal myomere counts for whitefish differed greatly from cisco, having a mode equal to the highest count attained by cisco (Fig. 14). The data indicates that fish with pre-anal myomere counts greater than 42 are very likely whitefish.

DORSAL PIGMENTATION

All whitefish examined showed an even distribution of melanophores over the entire dorsal surface from head to caudal fin (Figs. 15-17). This was apparent from time of hatching on. The melanophores were uniformly spaced along the entire surface with no clumping or increased denseness at the posterior end. The melanophores formed a double row in small fish and could appear paired or non-paired depending on degree of expansion or contraction (Fig. 15-16A(i)). Cisco smaller than approximately 18.0 mm S.L. showed a clumping (increased denseness) of dorsal melanophores in the posterior dorsal region and an uneven patchy distribution anteriorly (Fig. 158(i)). The dorsal pigment became more evenly distributed and similar to that of the whitefish for cisco larger than approximately 18.0 mm S.L. (Figs. 16-17). However, there still remained a tendency for the posterior dorsal surface of these larger cisco to appear to have a slightly increased density of melanophores when compared to whitefish of the same size (Fig. 16-17A & B(i)).

VENTRAL PIGMENTATION

All whitefish examined showed some anterior ventral pigmentation. Whitefish smaller than 18.0 mm S.L. were characterized by either a scattered array of melanophores (Fig. 15) or a mid-ventral line of melanophores. The latter pattern was quite common. Whitefish larger than 18.0 mm S.L. all showed only a mid-ventral line of melanophores (Figs. 16-17). This line always contained more than 15 pigment cells and usually more than 30. The mid-ventral line of melanophores tended to become fainter in larger whitefish (30 mm S.L.). Of the larval cisco examined, over 60% showed no anterior ventral pigmentation. Of those cisco that exhibited ventral pigmentation, 99% showed a scattered distribution of melanophores and the majority of these were smaller than 18.0 mm S.L. (Figs. 15-16). On cisco larger than 18.0 mm S.L., anterior ventral pigmentation was rare. Approximately 1% of larval cisco showed a faint mid-gut line of melanophores; however, in these fish the number of pigment cells was always less than 15 and usually less than 10 cells.

MOUTH POSITION

The mouthparts of some whitefish became identifiable as the typical inferior mouth (snout overhangs lower jaw) at approximately 20.0 mm S.L. (Fig. 17). However, it was variable until about 26.0 mm S.L. The mouthparts of some cisco became identifiable as the typical superior mouth (lower jaw extends beyond snout) at approximately 20.0 mm S.L. (Fig. 17). This was variable until 27.0 mm S.L. These pigmentation and morphological data are summarized and presented in a taxonomic dichotomous key format (Table 5).

ISOELECTRIC FOCUSING

Protein bands were distributed over the entire gel surface from pH 2.5-11.0, although most bands were located within the acidic pH range. The banding patterns are quite similar for the two species except at one location towards the acidic end of the gel (pH =5.0). The whitefish gels were characterized by three bands close together (dark, light, dark), commencing about 19 mm from the anode and extending over 2.5 mm along the length of the gel. The cisco gels were characterized by three more widely separated bands (dark, light, dark) representing different proteins (Fig. 18). They began about 19 mm from the anode and extended over 6.5 mm along the length of the gel.

The technique was tested on 89 larval whitefish (hatchery and wild stock), 94% of which showed the expected banding pattern and on 105 larval cisco (hatchery and wild stock), 85% of which showed the expected banding pattern (Table 6). All fish which exhibited banding showed the expected pattern. Fish which did not show the expected pattern did not show unexpected bands but rather showed an absence of bands (zero banding). Larval cisco were more susceptible to this phenomenon than whitefish. Hatchery reared larval whitefish and cisco, which were frozen directly, had a much lower frequency of zero banding than frozen wild caught specimens (2% and 6% vs 14% and 32%, respectively), which were usually caught in warm conditions and packed intermittently on ice for up to 6 hrs before freezing (Table 6). New extracts made from larval whitefish and cisco frozen for up to five years showed much less zero banding than old extracts, frozen for as little as 6 mon (5% and 12% vs 12% and 47%, respectively). I.E.F. done immediately on new extracts made from freshly caught fish showed no evidence of zero banding. The frequency of zero-banding was higher in non-eviscerated than eviscerated larval coregonids (Table 6). Although the digestive tract was a factor in zero-banding, its presence or absence did not determine the type of bands present. I.E.F. analysis of wild larval specimens indicated that 25 (85%) of 29 whitefish and 26 (69%) of 38 cisco showed the expected banding pattern. Of note is the fact that although the key to pigmentation was based upon examination of preserved larval fish (5% non-buffered formalin), it was successfully applied to wild larval fish captured and identified fresh, prior to performing I.E.F. The same key works well for preserved and fresh larvae, however, the increased body color and melanophore dilation/contraction of fresh larvae may cause initial problems of interpretation.

DISCUSSION

The great morphological similarity amongst larval forms of coregonids coupled with the plasticity of meristic characters such as fin ray counts, makes clear cut separation based upon them very difficult. Although adult forms of whitefish and cisco are identifiable through differences in both morphological and meristic characters (Lindsey et al. 1970; Scott and Crossman 1973), the larval forms were not easily separated using similar methods.

Measurements of various body parts (body depth, head length, eye diameter, pupil

diameter, pelvic fin length and pectoral fin length) demonstrated significant differences between mean values for whitefish and cisco. However, the plots of the various morphological characters versus standard length for both species indicated widely overlapping distributions. These overlaps limit the usefulness of these characters for definitive separation of species. No statistically significant differences occurred between the mean values for anal, caudal, dorsal fin ray counts and post-anal myomere counts of whitefish and cisco. The range of values exhibited by both whitefish and cisco for each of these meristic characters was identical, invalidating their use as mechanisms of species delineation. Statistically, significant differences were evident for pre-anal myomere counts. Whitefish showed higher mean pre-anal myomere counts and a range of values which exceeded that for cisco by two. This character can be utilized in the identification procedure.

It has long been noted and demonstrated that larval coregonids can exhibit different pigmentation patterns useful for species identification (Lindstrom 1962; Faber 1970; Hinrichs 1979; Cucin and Faber 1985). Faber (1970) utilized dorsal pigmentation patterns in conjunction with pre- and post-anal myomere counts and spatial distribution to separate C. clupeaformis and C. artedii at the early post hatching stage. Considerable variation in all the above characters rendered them inadequate to allow easy identification of the larvae. This study did not investigate the changing pigmentation patterns of the larvae at increased growth stages. Hinrichs (1979) described seven embryonic and six larval stages for five coregonid species. The pigmentation was described at each stage. Separation of C. artedii from C. clupeaformis was based primarily on mouth position, and two ratio measurements (dorsal fin origin to snout ÷ standard length and diameter of largest dorsal melanophore ÷ width of myomere). Work by Cucin and Faber (1985) suggests that this latter relationship between melanophore diameter and myomere width would not always be valid. In their study, the relationship between incident light and concentration or dilation of melanophores on live lake whitefish was investigated. They found that in conditions of zero incident light the melanophores became small concentrated dots (fish appear light), and the degree of melanophore concentration increased directly with time in complete darkness. In their own key to separation, Cucin and Faber (1985) described C. clupeaformis up to 15 mm S.L. as having an even double row of dorsal melanophores and a row of non-uniform lateral melanophores and C. artedii up to 11.5 mm S.L. as having an incomplete or uneven double row of dorsal melanophores and a row of uniform lateral melanophores. In their study, only early post-hatching larval fish were described because the sampling procedure did not catch larger specimens. Thus, this key, although working well for small larval fish, does not allow for changing pigmentation patterns at advanced larval stages. Johnston (1984) also found that larval lake whitefish showed a contraction of the melanophores and a pale appearance in response to complete darkness. However, in situations of reduced incident light or dark colored background fish appear dark (expanded melanophores) and fish appeared lighter (contracted melanophores), as incident light increased or in light colored background. The study also determined that larval whitefish can produce more melanophores in response to long term reductions in incident light. Since whitefish are mainly bottom feeders (dark colored background) and cisco are pelagic zooplanktivores (light colored background) (Scott and Crossman 1973), one would expect some predisposition to more body melanophores and darker appearance in the whitefish. Johnston (1984) was able to demonstrate separation of two sympatric color morphs of whitefish on the basis of actual melanophore count. However, since actual mean counts were quite similar, it was found that the degree of expansion or contraction of melanophores could lead to misinterpretation if fish color was assessed qualitatively. Thus, to avoid the problems of melanophore plasticity in both size and number, qualitative and quantitative pigmentation differences should be utilized for species identification only when such differences are large.

Different pigmentation characters (dorsal and ventral) proved to be useful for the separation of whitefish and cisco over a fairly broad range of sizes, enabling the use of multiple parameters in the identification decision. The best method for identifying small coregonid larvae was the dorsal pigmentation where whitefish had a distinct even double row of melanophores and cisco had melanophores concentrated on the posterior dorsal surface. This characteristic appears similar to that described by Cucin and Faber (1985). With larger larvae (18 mm S.L.), where the dorsal pigmentation of cisco appeared similar to that of whitefish, the anterior ventral pigmentation could be used as an identification character. Whitefish always showed ventral pigmentation. On whitefish larger than 18 mm S.L. this pigment was always in the form of a mid gut line of 15 melanophores. Cisco, by and large, exhibited minimal anterior ventral pigmentation, especially those larger than 18 mm S.L. Of those cisco with ventral pigmentation, the majority exhibited a scattered array of melanophores. The rest (≈1%) showed the mid gut line of melanophores seen in whitefish; however, the number of pigment cells on this mid gut line in these individuals was always less than 15. Illustrations in Cucin and Faber (1985) show the cisco as having a scattered array of melanophores on the anterior gut and the lake whitefish as having a mid gut line of melanophores. This suggests that pigmentation patterns for C. clupeaformis and C. artedii from central Ontario may be similar to those found in fish from northern Manitoba. Differences in mouth shape and position were important identification features in larger larval fish (≈26 mm S.L.).

Although the separation of larval whitefish and cisco based on pigmentation and mouth position differences was shown to work extremely well for both preserved stock and wild caught specimens, some further absolute test was considered essential. Hence, the search for a genetic protein marker to flag the species difference. Various researchers have had success separating both unrelated and closely related species of marine fish on the basis of isoelectric focusing of sarcoplasmic proteins

(Lundstrom 1979, 1980; Mackie and Ritchie 1981). Since it is extremely difficult to obtain enough material of one organ or tissue type from one larval fish and since the mixing of tissues from several fish would not allow the identification of individual fish, it was deemed best to macerate eviscerated whole larval fish. This method led to a separation technique, although it is unknown which tissues or organ type led to the protein differences.

Although a high percentage of larval coregonid I.E.F. analyses produced the expected species pattern, a small fraction showed no protein banding at all. The zero banding was likely brought on by protein de-naturation. Our results indicate that poor initial handling of collected specimens and long term storage of prepared extracts led to a higher incidence of the problem. Non-evisceration of the larval specimens also tended to increase zero banding perhaps through enhanced decay of gut contents and ultimately the organism. Similarly, King and Moffett (1985) found that inadequate handling or prolonged storage of extracts led to a general loss of protein bands on I.E.F. gels. In their study, duplicate I.E.F. runs were performed on extracts derived from two subsamples of Atlantic herring. One group of fish was handled properly (iced and promptly frozen) the other was allowed to sit at ≈13°C for 10 hrs. The gels derived from extracts from improperly handled fish showed significant reduction in all major protein bands and more importantly the loss of a major band which was present in gels derived from properly handled fish. This situation could lead to misinterpretations concerning species identification and, in their study, discrimination of stocks. It is imperative that extreme care be given to wild captured fish specimens (icing and prompt freezing). In our study this potential for misinterpretation has been accounted for and we feel that the observed protein banding patterns represent a real difference between species not artifacts. In conventional I.E.F. of large fish, 1.0 g of flesh is used. In our study, the total larval fish may weigh only 0.1-0.4 g, hence we tended to see an absence rather than simply a reduction of protein activity. Future work on this problem might involve re-analyzing fish samples having zero banding and utilizing the more sensitive silver stain technique instead of Coomassie blue. Attempts were made to overstain gels already stained in Coomassie blue R-250 with the silver stain. These met with limited success as the gels became too dark to discriminate individual bands.

The I.E.F. method could be streamlined by narrowing the pH range of the gel to encompass only the area of note at the acidic end. The focusing could then proceed across the gel with up to 25 fish per run. Although the procedure is not highly dependent on I.E.F conditions (time and applied power), care should be exercised to ensure a standard sample size application technique. Variations could apply more or less protein and possible intensity variations could occur in the banding patterns.

In this study, larval lake whitefish and cisco from SIL have been successfully identified on the basis of differences in body pigmentation patterns, mouth shape and biochemically through protein variation. The technique allows for quick field identification and a positive laboratory check on the efficiency of species identification. These methods of identification work well for fish from the Southern Indian Lake system. They may not hold true or be entirely valid when dealing with other systems. Reports by Faber (1970) and Reckahn (1970) indicate that larval whitefish and cisco from Lake Huron may exhibit different patterns of body pigmentation than SIL coregonids. Illustrations in Cucin and Faber (1985), although indicating pigmentation pattern similarities between coregonids from central Ontario and northern Manitoba, do so for only one larval size and may not hold at more advanced stages. It is also possible that the biochemical markers found in SIL coregonids might not be found in fish from other areas. The methods are as yet untested on larval specimens outside SIL.

In studies investigating the larvae of morphologically similar symnatric species of coregonids, the approach to identification of the larvae should involve culturing known specimens of the species, formation of keys based on morphological research of cultured specimens, and finally, identification of a biochemical marker as a check on the validity of morphological keys.

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REFERENCES

AMEND, D.F. 1974. Comparative toxicity of two Iodophors to rainbow trout eggs. Trans. Am. Fish. Soc. 103: 73-78.

BISHOP, R. 1979. Current major application areas in electrofocusing. Science Tools 26(1): 2-8.

CHELLEVOLD, R.J. 1970. The genetic relationships of intergeneric and intergeographical coregonids as determined by protein properties. p. 115-126. In C.C. Lindsey and C.S. Woods (ed.) Biology of coregonid fishes. University of Manitoba Press, Winnipeg, MB.

CUCIN, D., and D.J. FABER. 1985. Early life studies of lake whitefish (Coregonus clupeaformis), cisco (Coregonus artedii) and yellow perch (Perca flavescens) in Lake Opeongo, Ontario. Ont. Fish. Tech. Rep. Ser. 16: iii + 28 p.

DJUPSUND. B.M. 1976. Proteintaxonomical studies of whitefish and tapeworms with thin layer electrofocusing. (LKB application note 243) - LKB - producter ABS-161 25, Bromma. Sweden.

FABER, D.J. 1970. Ecological observations on newly hatched lake whitefish in South Bay, Lake Huron, p. 481-500. In C.C. Lindsey and C.S. Woods (ed.) Biology of coregonid fishes. University of Manitoba Press, Winnipeg, MB.

FABER. D.J., and S. GADD. 1983. Several drawing techniques to illustrate larval fishes. Trans. Am. Fish. Soc. 112: 349-353.

FISH. M.P. 1932. Contributions to the early life histories of sixty-two species of fishes from Lake Erie and its tributary waters. Bull. U.S. Bur. Fish. 10: 293-398.

HINRICHS, M.A. 1979. A description and key of the eggs and larvae of five species of fish in the subfamily Coregoninae. M.Sc. Thesis. University of Wisconsin. Stevens Point, WI. 73 p.

JOHNSTON, C.H. 1984. The genetic and environmental basis for external coloration in lake whitefish (Coregonus clupeaformis (Mitchill)) from Southern Indian Lake, Manitoba. M.Sc. Thesis, University of Manitoba, Winnipeg, 132 p.

KING, D.P.F., and I.J.J. MOFFETT. 1985. Evaluation of isoelectric focusing of sarcoplasmic proteins as a means of separating stocks of Atlantic herring (Clupea harengus L.) in the North Irish Sea. Rec. Agric. Res. (Belfast) 32(0): 103-120.

LEITRITZ, E. 1959. Trout and salmon culture (hatchery methods). Calif. Dep. Fish Game Bull. 107: 155 p.

LINDSEY, C.C., J.W. CLAYTON, and W.G. FRANZIN. 1970. Zoogeographic problems and protein variation in the Coregonus clupeaformis whitefish species complex, p. 127-146. In C.C. Lindsey and C.S. Woods (ed.) Biology of coregonid fishes. University of Manitoba Press, Winnipeg, MB.

LINDSTROM, T. 1962. Life history of whitefish young (Coregonus) in two lake reservoirs. Rep. Inst. Freshwater Res. Drottningholm 44: 113-144.

LIPPSON, A.J. 1976. Distinguishing family characteristics among Great Lakes fish larvae. (App. 3 of Great Lakes fish egg and larvae identification). National Power Plan Project. Office of Biological Services, U.S. Fish and Wildlife Service.

LUNDSTROM, R.C. 1979. Fish species identification by thin layer isoelectric focusing. J. Assoc. Off. Anal. Chem. 62(3): 624-629.

LUNDSTROM, R.C. 1980. Fish species identification by thin layer polyacrylamide gel isoelectric focusing: collaborative study. J. Assoc. Off. Anal. Chem. 63(1): 69-73.

LUNDSTROM, R.C. 1981a. Fish species identification by isoelectric focusing: sacroplasmic protein polymorphism in monkfish (Lophius americanus). J. Assoc. Off. Anal. Chem. 64(1): 32-37.

LUNDSTROM, R.C. 1981b. Rapid fish species identification by agarose gel isoelectric focusing of sarcoplasmic proteins. J. Assoc. Off. Anal. Chem. 64(1): 38-43.

LUNDSTROM, R.C., and S.A. RODERICK. 1979. Fish species identification by thin-layer isoelectric focusing of sarcoplasmic proteins. Science Tools, 26(3): 38-43.

MACKIE, I.M., and A.H. RITCHIE. 1981. Differentiation of Atlantic cod Gadus morhua morhua and Pacific cod Gadus morhua macrocephalus by electrophoresis and by isoelectric focusing of water-soluble proteins of muscle tissue. Comp. Biochem. Physiol. 68B: 173-175.

PRITCHARD, A.L. 1930. Spawning habits and fry of the cisco Leucichthys artedii in Lake Ontario. Contrib. Can. Biol. Fish. (NS) 6: 225-240.

RECKHAN, J.A. 1970. Ecology of young lake whitefish (Coregonus clupeaformis) in South Bay, Manitoulin Island, Lake Huron, p. 437-460. In C.C. Lindsey and C.S. Woods (ed.) Biology of coregonid fishes. University of Manitoba Press, Winnipeg, MB.

SCOTT, W.B., and E.J. CROSSMAN. 1973. Freshwater fishes of Canada. Fish. Res. Board Can. Bull. 184. 966 p.

SNYDER, D.E. 1976. Terminologies for intervals of larval development. In J. Boreman (ed.) Great Lakes fish egg and larvae identification. Proceedings of a workshop. National Power Plant Team. U.S. Fish and Wildlife Service, Ann Arbor, MI.

VUORINEN, J., M.K.-J. HIMBERG, and P. LANKINEN. 1981. Genetic differentiation in Coregonus albula (L.) (Salmonidae) populations in Finland. Hereditas 94: 113-121.

VUORINEN, J., and J. PIIRONEN. 1984. Electrophoretic identificaton of Atlantic salmon (Salmo salar). brown trout (S. trutta) and their hybrids. Can. J. Fish. Aquat. Sci. 41(12): 1834-1837.

WOOD, E.M., and W.A. DUNN. 1948. Fact and fiction in spawntaking. Prog. Fish. Cult. 10: 67-72.

YAMADA, J., and A. SUZUKI. 1982. Identification of fish species by thin layer isoelectric focusing of sacroplasmic protein. Bull. Jpn. Soc. Sci. Fish. 48: 73-77.

Table 1. Formula for standard gel utilized in isoelectric focusing.

4.0 mL of 30.5% Acrylamide
4.0 mL of 1.0% Bis-acrylamide
12.0 mL of 10.0 M Urea
0.08 mL of 5.0% Dithioerythritol
1.8 mL of carrier ampholytes
- 0.8 mL pH 3.5-10
- 0.2 mL pH 2.5-4
- 0.1 mL pH 3.5-5
- 0.1 mL pH 4-6
- 0.1 mL pH 5-7
- 0.1 mL pH 6-8
- 0.1 mL pH 7-9
- 0.1 mL pH 8-9.5
- 0.2 mL pH 9-11
Degas solution for 2 min
1.2 mL of 10% Triton + (nonionic detergent)
1.4 mL of 0.004% riboflavin

Table 2. Summary of morphological and meristic measurements by length class for hatchery reared lake whitefish and cisco from Southern Indian Lake.

Species	Whitefish						Cisco						Whitefish						Cisco					
Characters	n	Maximum	Minimum	Mean	Variance	Standard Deviation	n	Maximum	Minimum	Mean	Variance	Standard Deviation	n	Maximum	Minimum	Mean	Variance	Standard Deviation	n	Maximum	Minimum	Mean	Variance	Standard Deviation
Standard length	10.0-15.0 (mm)												15.1-20.0 (mm)											
Total length	10.8-15.7 (mm)												15.6-23.0 (mm)											
Body depth (mm)	49	2.02	1.16	1.53	0.03	0.19	47	1.84	0.90	1.21	0.04	0.21	26	3.60	1.40	2.32	0.40	0.63	8	3.08	2.01	2.41	0.16	0.40
Head depth (mm)	47	2.01	1.40	1.75	0.02	0.15	47	1.88	1.22	1.47	0.03	0.16	26	3.00	1.67	2.42	0.16	0.40	8	2.80	2.12	2.32	0.06	0.24
Head length (mm)	49	3.12	2.08	2.52	0.06	0.24	47	2.92	1.62	2.10	0.08	0.29	26	5.00	2.62	3.74	0.47	0.70	8	4.24	3.10	3.65	0.20	0.44
Eye diameter (mm)	49	1.88	0.92	1.10	0.02	0.15	47	1.12	0.72	0.90	0.01	0.09	26	1.80	1.20	1.44	0.03	0.16	8	1.54	1.24	1.37	0.01	0.11
Pupil diameter (mm)	49	0.45	0.24	0.35	0.00	0.05	47	0.44	0.28	0.33	0.00	0.04	26	0.61	0.41	0.52	0.01	0.07	8	0.60	0.44	0.49	0.00	0.06
Pelvic fin length (mm)	16	0.58	0.24	0.39	0.12	0.11	9	0.52	0.20	0.36	0.01	0.10	25	1.60	0.48	0.91	0.11	0.34	8	1.12	0.3	0.71	0.05	0.23
Pectoral fin length (mm)	49	2.20	1.20	1.89	0.05	0.21	47	2.12	1.22	1.64	0.04	0.20	24	2.90	2.04	2.44	0.07	0.26	7	2.60	2.10	2.25	0.03	0.18
Dorsal fin rays	23	10.00	6.00	7.83	2.51	1.59	11	11.00	1.00	7.00	7.60	2.76	26	12.00	7.00	10.69	1.34	1.16	8	12.00	10.00	10.75	0.50	0.72
Anal fin rays	9	9.00	5.00	7.44	1.78	1.33	8	10.00	4.00	7.00	4.57	2.14	22	12.00	7.00	10.55	2.36	1.54	8	12.00	10.00	10.88	0.70	0.84
Caudal fin rays	47	18.00	1.00	10.30	20.65	4.54	39	19.00	3.00	8.03	25.87	5.09	26	24.00	12.00	20.73	9.81	3.13	8	24.00	18.00	22.38	4.28	2.07
Post-anal myomeres	49	20.00	10.00	17.31	3.01	1.74	47	20.00	13.00	17.40	2.59	1.61	21	18.00	14.00	15.38	1.05	1.02	7	18.00	14.00	16.00	2.67	1.63
Pre-anal myomeres	49	43.00	39.00	40.53	1.17	1.08	47	41.00	35.00	38.66	1.19	1.09	26	43.00	39.00	41.58	1.13	1.07	8	41.00	39.00	39.75	0.79	0.89
Standard length	20.1-25.0 (mm)												25.1-32.0 (mm)											
Total length	23.0-29.0 (mm)												28.8-38.0 (mm)											
Body depth (mm)	23	4.46	2.88	3.84	0.23	0.48	14	4.80	2.80	3.80	0.34	0.59	11	6.72	4.57	5.24	0.35	0.59	15	6.57	4.60	5.42	0.44	0.66
Head depth (mm)	23	4.04	2.80	3.44	0.10	0.32	14	3.92	2.72	3.37	0.10	0.32	11	5.14	4.00	4.44	0.10	0.32	14	5.00	3.60	4.30	0.19	0.43
Head length (mm)	23	6.43	4.60	5.46	0.26	0.51	14	5.29	4.24	5.06	0.31	0.55	11	8.71	6.35	7.17	0.51	0.71	15	8.57	5.20	6.69	1.07	1.03
Eye diameter (mm)	23	2.20	1.64	1.91	0.02	0.14	14	2.00	1.52	1.74	0.02	0.14	11	2.67	2.25	2.39	0.02	0.13	15	2.57	1.88	2.20	0.04	0.21
Pupil diameter (mm)	23	0.87	0.59	0.73	0.01	0.07	14	0.73	0.55	0.62	0.00	0.06	11	1.13	0.80	0.94	0.01	0.10	15	1.00	0.68	0.80	0.01	0.11
Pelvic fin length (mm)	23	2.88	1.60	2.12	0.13	0.36	14	2.52	1.04	1.82	0.22	0.47	6	4.43	1.80	3.22	0.73	0.85	14	3.73	2.16	2.88	0.18	0.42
Pectoral fin length (mm)	21	3.28	1.80	2.77	0.15	0.38	12	3.20	2.00	2.70	0.17	0.42	3	3.80	3.47	3.68	0.03	0.19	14	4.16	2.73	3.41	0.18	0.43
Dorsal fin rays	23	14.00	11.00	12.04	6.68	2.59	14	13.00	11.00	11.64	0.56	0.75	11	14.00	13.00	13.27	0.22	0.47	15	14.00	11.00	12.80	1.03	1.01
Anal fin rays	23	16.00	10.00	12.70	1.86	1.36	14	15.00	11.00	13.07	1.15	1.07	11	15.00	12.00	13.55	0.87	0.93	15	15.00	13.00	14.20	0.31	0.56
Caudal fin rays	22	33.00	22.00	27.91	8.94	2.99	12	33.00	24.00	28.25	9.48	3.08	11	43.00	30.00	33.55	13.67	3.70	14	42.00	29.30	35.86	11.21	3.35
Post-anal myomeres	22	16.00	13.00	14.41	0.73	0.85	13	16.00	14.00	14.77	0.36	0.60	9	15.00	13.00	14.00	0.25	0.50	15	28.00	14.00	14.87	1.41	1.19
Pre-anal myomeres	22	43.00	38.00	41.36	2.15	1.47	14	40.00	37.00	39.57	0.73	0.85	10	42.00	39.00	40.50	0.72	0.85	15	41.00	38.00	39.67	0.67	0.82

Table 3. Analysis of covariance for morphometric characters of hatchery reared lake whitefish and cisco from Southern Indian Lake. Standard length is the independent variable. (* symbol indicates significance at $p \leq 0.05$ level.)

Morphometric character	Slope (regression coef.)			Adjusted means		
	Whitefish	Cisco	Sig.F.	Whitefish	Cisco	Sig.F.
Body depth	0.260	0.267	N.S.	2.51	2.61	*
Head depth	0.188	0.184	N.S.	2.48	2.44	N.S.
Head length	0.326	0.297	*	3.81	3.67	*
Eye diameter	0.091	0.084	*	1.45	1.35	*
Pelvic fin length	0.205	0.192	*	1.56	1.39	*
Pectoral fin length	0.110	0.110	N.S.	2.27	2.16	*
Pupil diameter	0.040	0.030	*	0.520	0.490	*

Table 4. Analysis of variance for meristic characters of lake whitefish and cisco from Southern Indian Lake. Probability of equality of least squares means (adjusted for effects of interspecific size variation).

Meristic character	Cisco		Lake whitefish		Significance (Prob.>ITI Ho: L.S.MI = L.S.M2
	Observed x	L.S. mean	Observed x	L.S. mean	
Dorsal finrays	10.79	10.71	10.61	10.72	0.98 N.S.
Anal finrays	11.98	6.50	11.39	8.09	0.08 N.S.
Caudal finrays	18.26	18.05	18.92	18.87	0.62 N.S.
Post-anal myomeres	16.40	16.46	15.98	15.98	0.09 N.S.
Pre-anal myomeres	39.07	39.12	40.95	40.95	0.0001* Sig.

Table 5. Key to the identification of lake whitefish (C. clupeaformis) and cisco (C. artedii) from Southern Indian Lake.

Key to subfamily Coregoninae (Lippson 1976)

Yolk-sac larvae: large; less robust than salmons and trouts; head large; yolk absorbed before fin development; vent ca. 2/3 back on body; dorsal finfold with two indentations.

Larvae: robust; head rounded; adipose fin; pelvic fin origin below dorsal fin; anal fin origin well posterior to dorsal fin base.

1. (a) Dorsal melanophores evenly distributed along entire surface with no indication of increased concentration independent of degree of melanophore dilation of contraction 2
   (b) Dorsal melanophores concentrated posteriorly, with either a patchy uneven distribution of or absence of anterior melanophores ..... Coregonus artedii (Cisco).
2. (a) Larva <16.0 mm standard length (S.L.) ..... Coregonus clupeaformis (lake whitefish).
   (b) Larva >16.0 mm S.L. 3
3. (a) Anterior ventral melanophores present, forming a distinct mid-gut line. 4
   (b) No anterior ventral melanophores present or melanophores scattered randomly over gut ..... Coregonus artedii.
4. (a) Melanophores forming a mid-gut line of >15 melanophore cells (usually >30); pre-anal myomere count 38-43; mouth inferior (upper jaw overhangs lower jaw, trait is variable from 20-26 mm S.L.) ..... Coregonus clupeaformis.
   (b) Melanophores forming a mid-gut line of <15 melanophore cells (usually 6-8); pre-anal myomere count 35-41; mouth superior (lower jaw extends beyond upper jaw, trait is variable from 20-26 mm S.L.) ..... Coregonus artedii.

Note: Length cutoffs above indicate minima. Larval traits outlined can operate over a broader range of lengths and should be looked for. I.E.F. should be used to confirm efficiency or to identify individuals which may not fit into the key.

Table 6. Summary of isoelectric focusing analyses done on larval lake whitefish and cisco from Southern Indian Lake.

	Total no. larvae	No. hatchery reared larvae	No. wild larvae	No. new extracts from larvae frozen (>6 mo)	No. old extracts made and frozen (>6 mo)	No. new extracts from fresh larvae	No. larvae eviscerated	No. larvae non-eviscerated
Whitefish								
- Total sample	89	60	29	60	17	12	62	27
- No. showing expected	84	59	25	57	15	12	61	23
- % showing expected	94	98	86	95	88	100	98	85
- % showing zero banding	6	2	14	5	12	0	2	15
Cisco								
- total sample	105	67	38	78	15	12	52	53
- No. showing expected	89	63	26	69	8	12	47	42
- % showing expected	85	94	68	88	53	100	90	79
- % showing zero banding	15	6	32	12	47	0	10	21

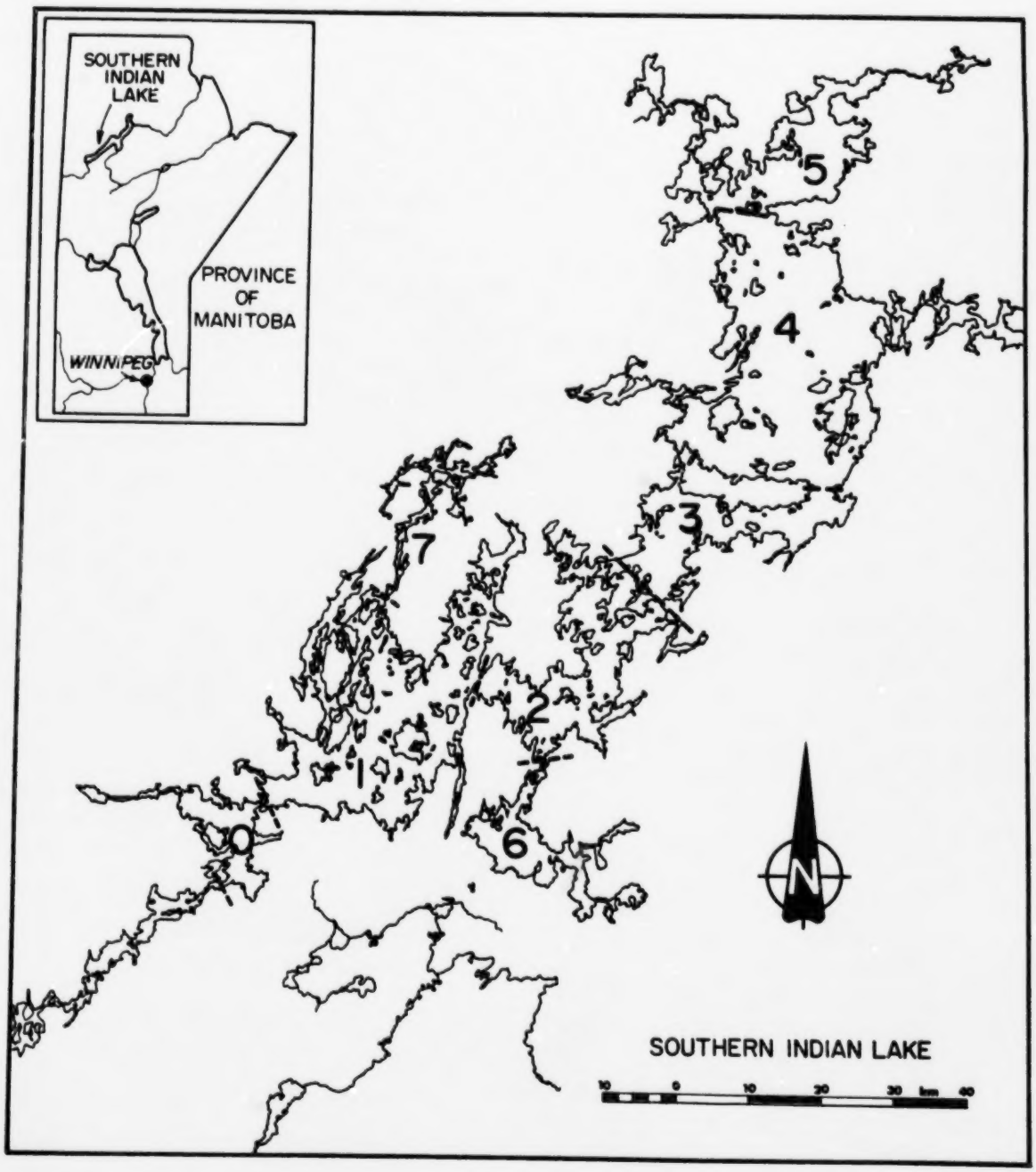

Fig. 1. Location of Southern Indian Lake in Manitoba showing eight major basins of the lake delineated for limnological studies.

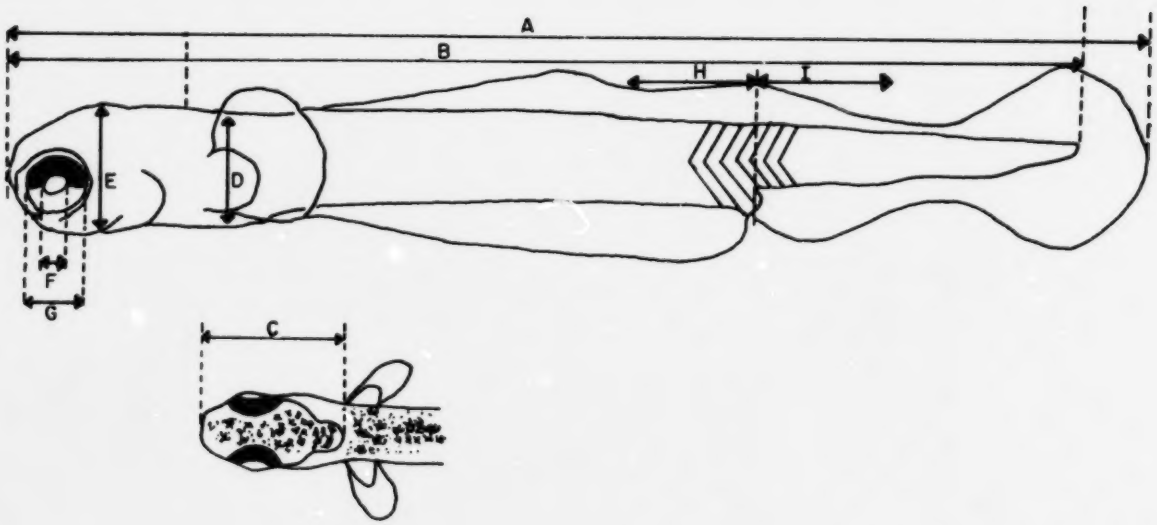

Fig. 2. Standard morphological measurements done for hatchery reared lake whitefish and cisco from Southern Indian Lake: (A) Total Length (B) Standard (Notochord) Length (C) Head Length (D) Body Depth (E) Head Depth (F) Pupil Diameter (G) Eye Diameter (H) Pre-Anal Myomeres (I) Post Anal Myomeres.

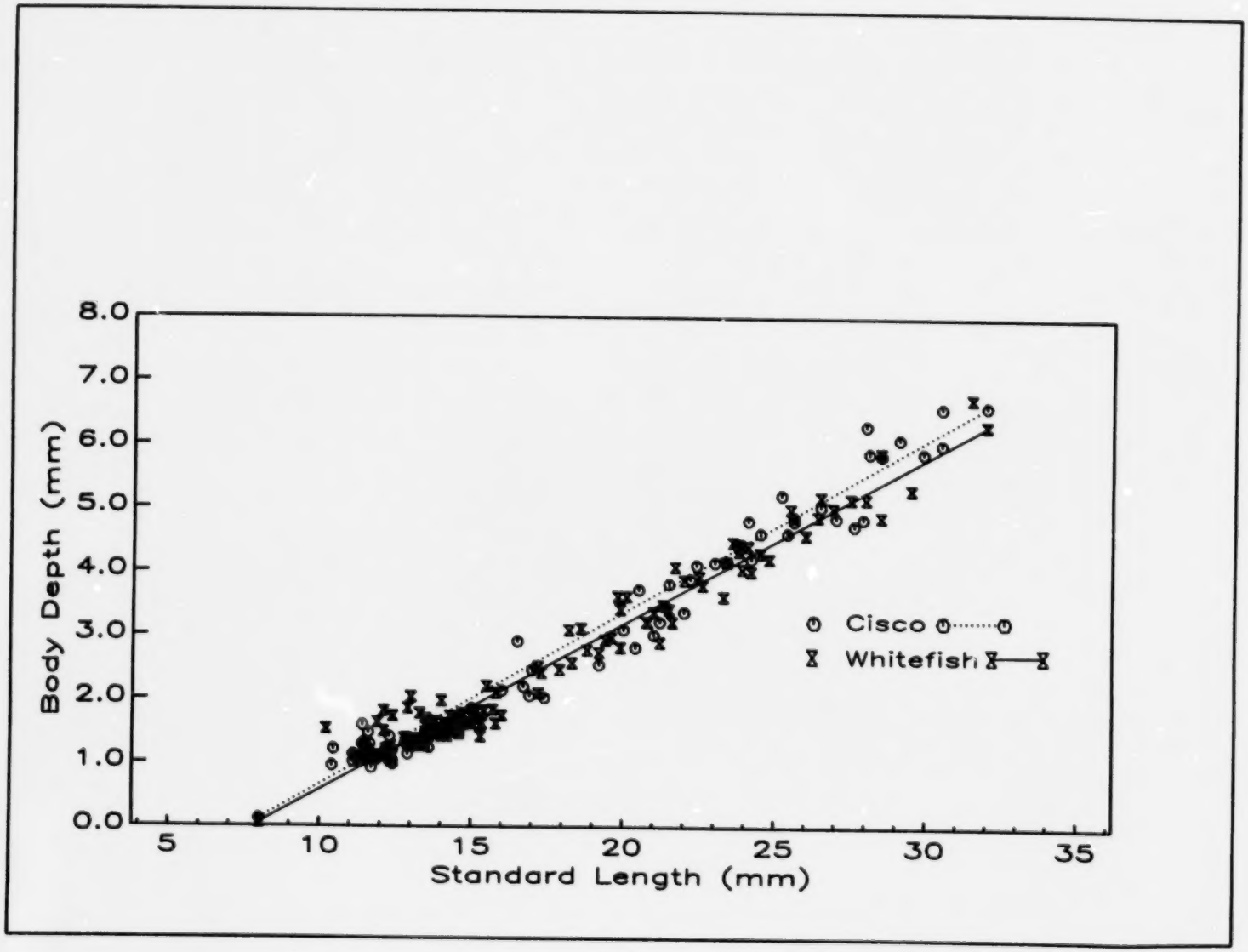

Fig. 3. Body depth versus standard length for hatchery reared lake whitefish and cisco.

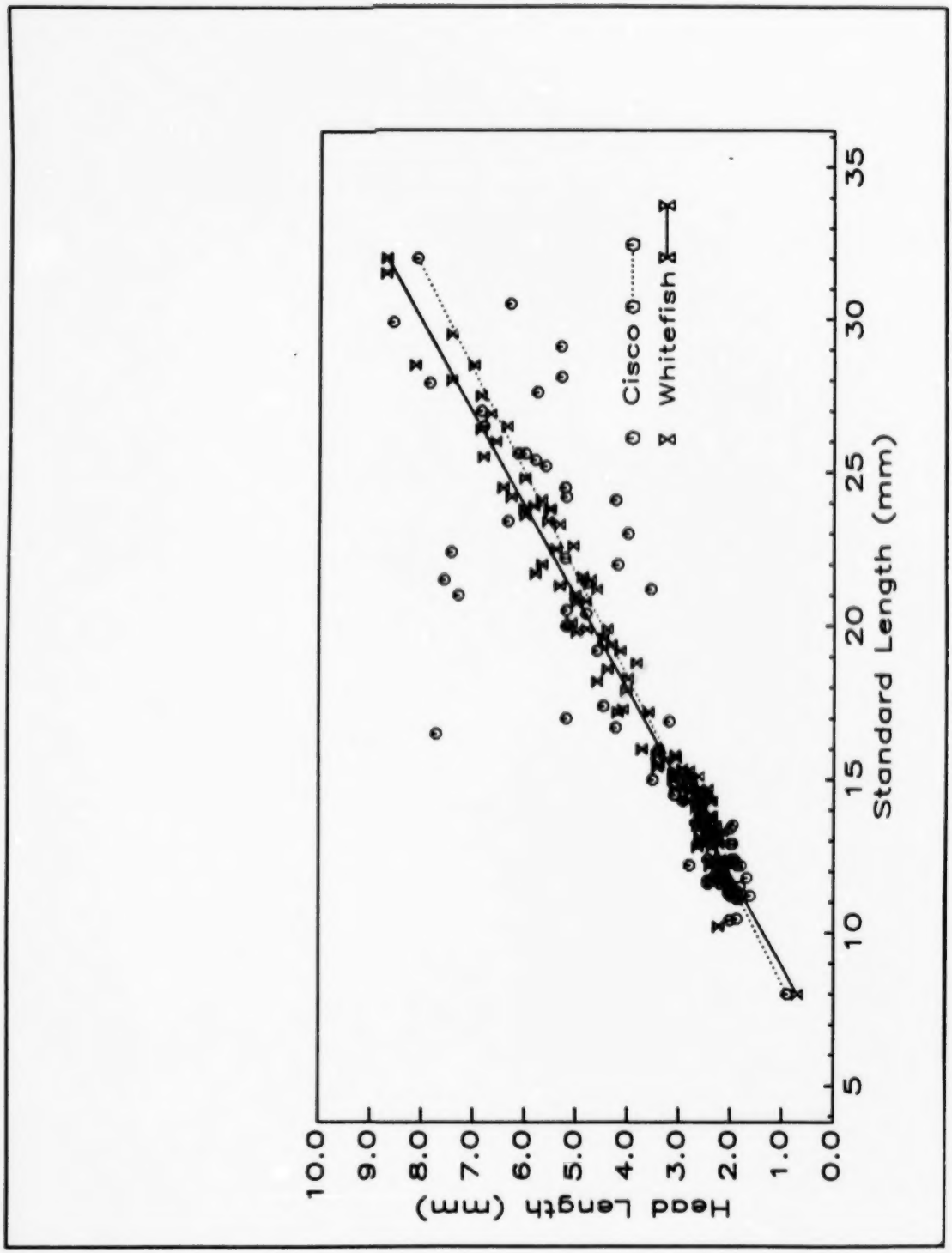

Fig. 4. Head length versus standard length for hatchery reared lake whitefish and cisco.

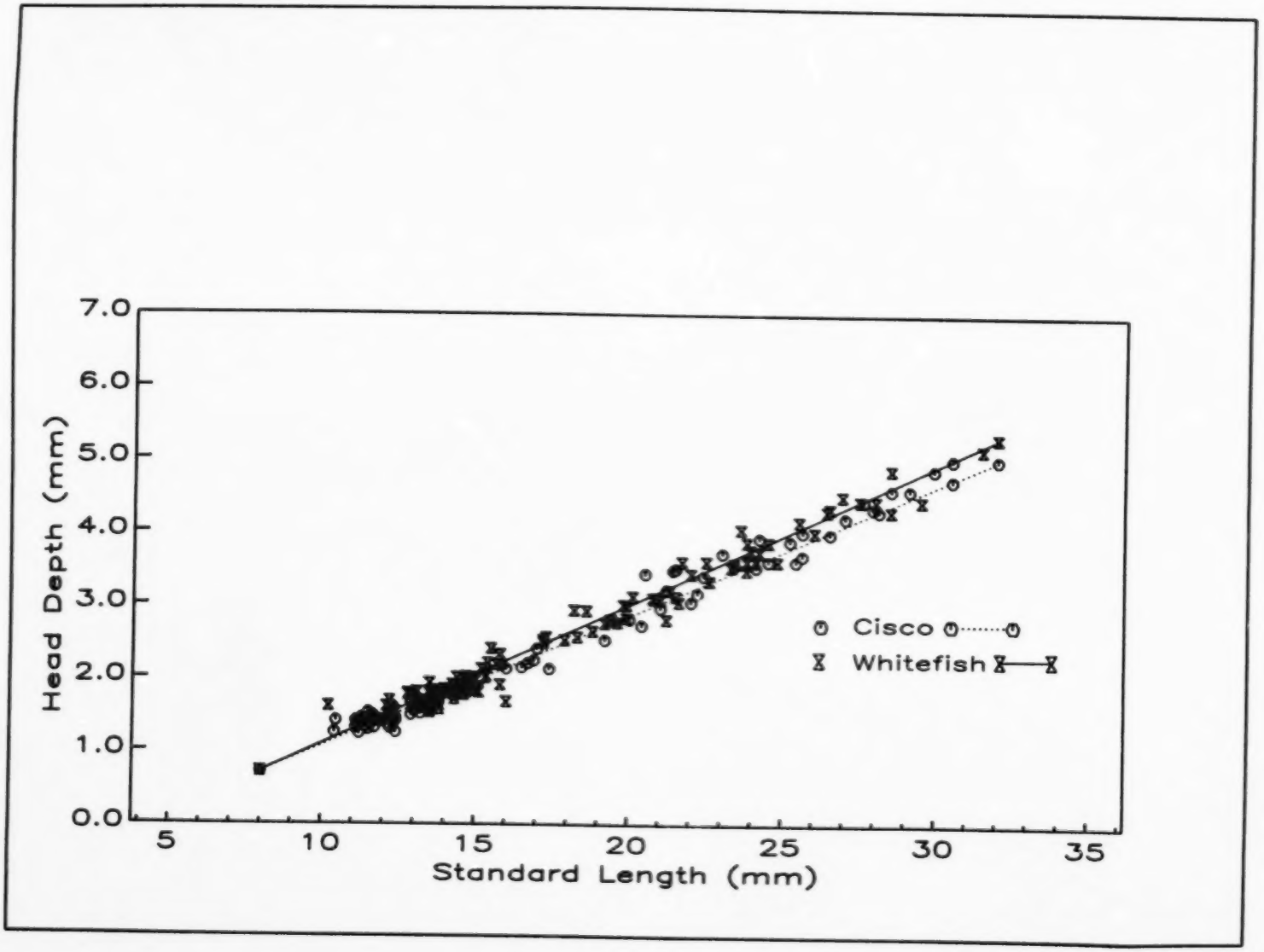

Fig. 5. Head depth versus standard length for hatchery reared lake whitefish and cisco.

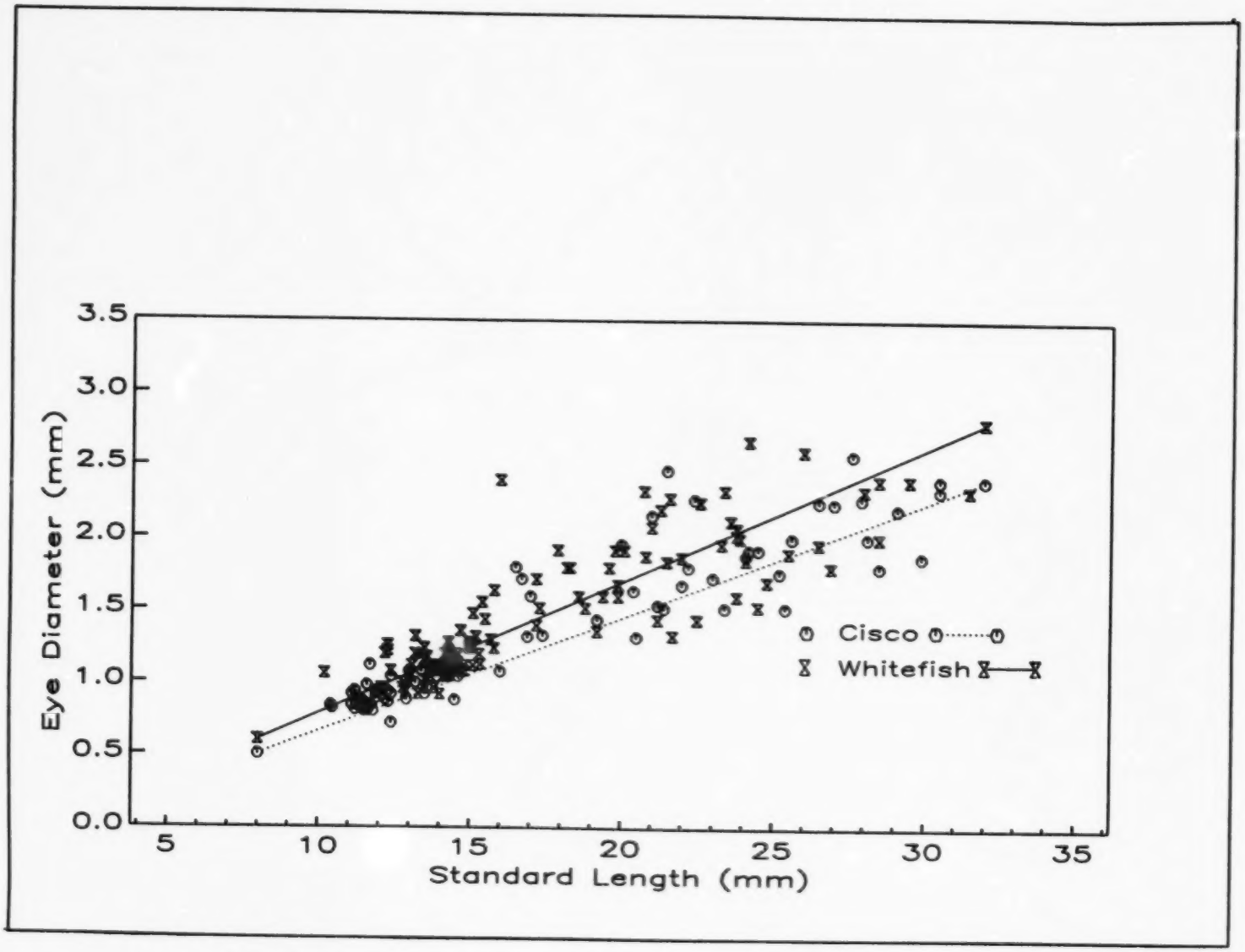

Fig. 6. Eye diameter versus standard length for hatchery reared lake whitefish and cisco.

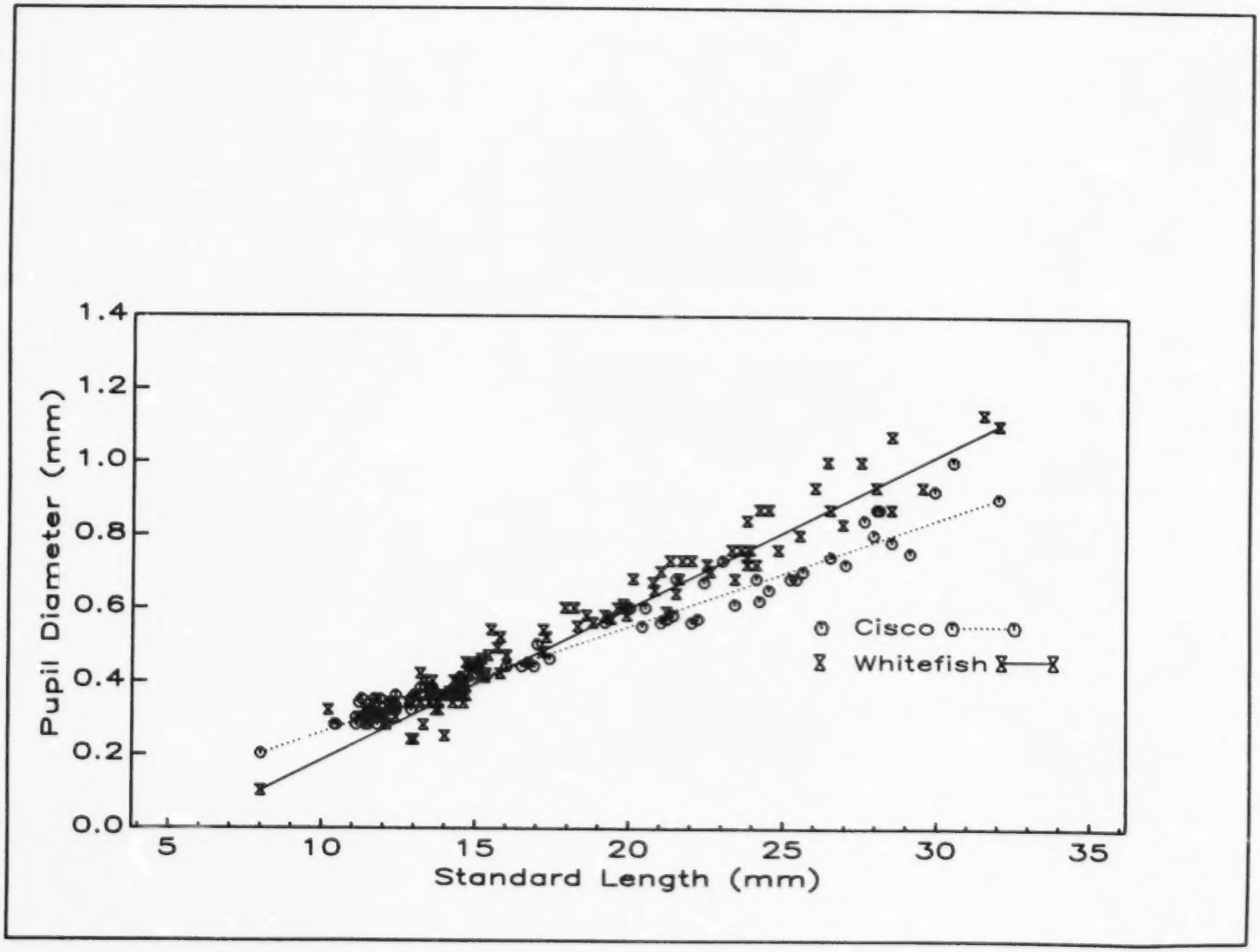

Fig. 7. Pupil diameter versus standard length for hatchery reared lake whitefish and cisco.

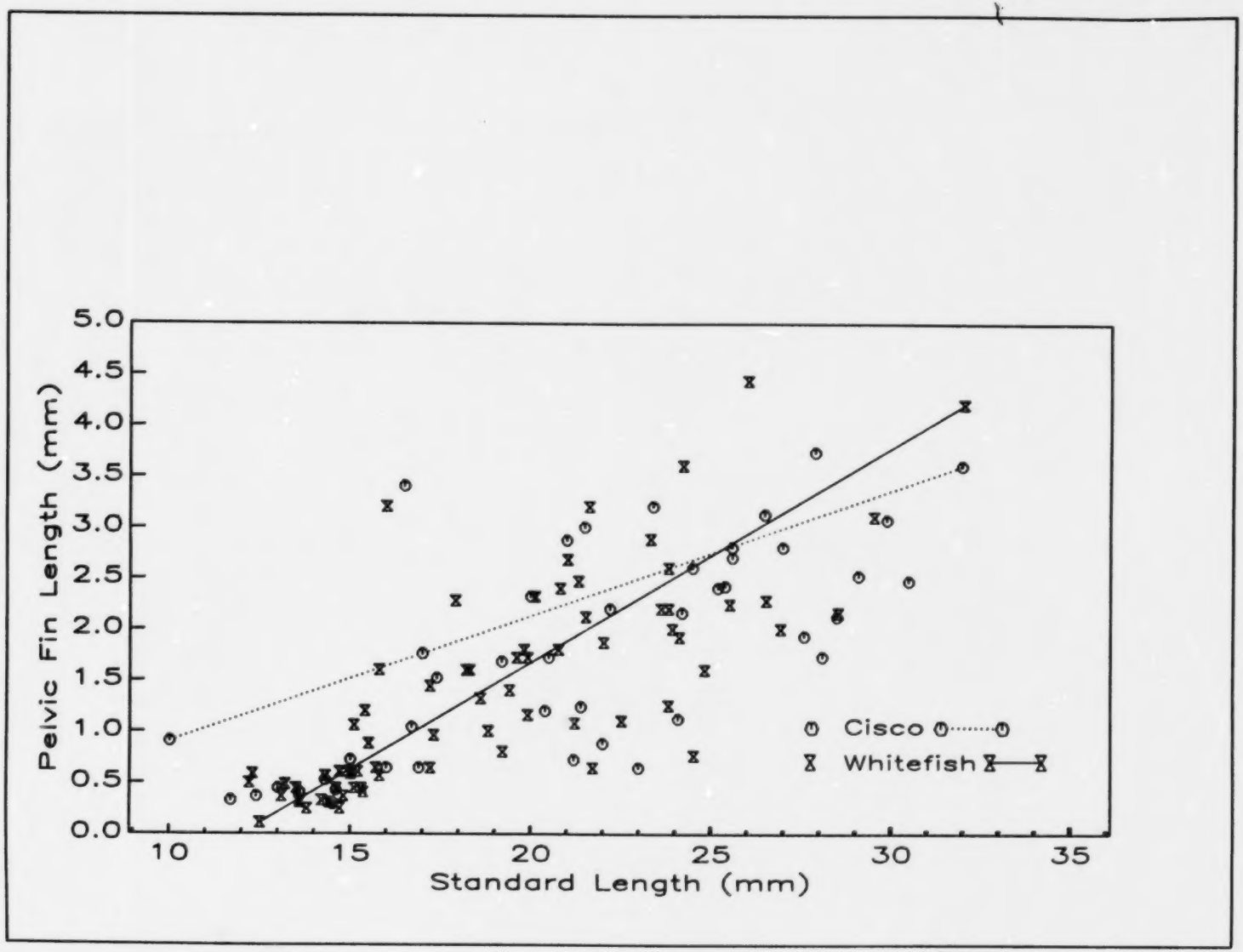

Fig. 8. Pelvic fin length versus standard length for hatchery reared lake whitefish and cisco.

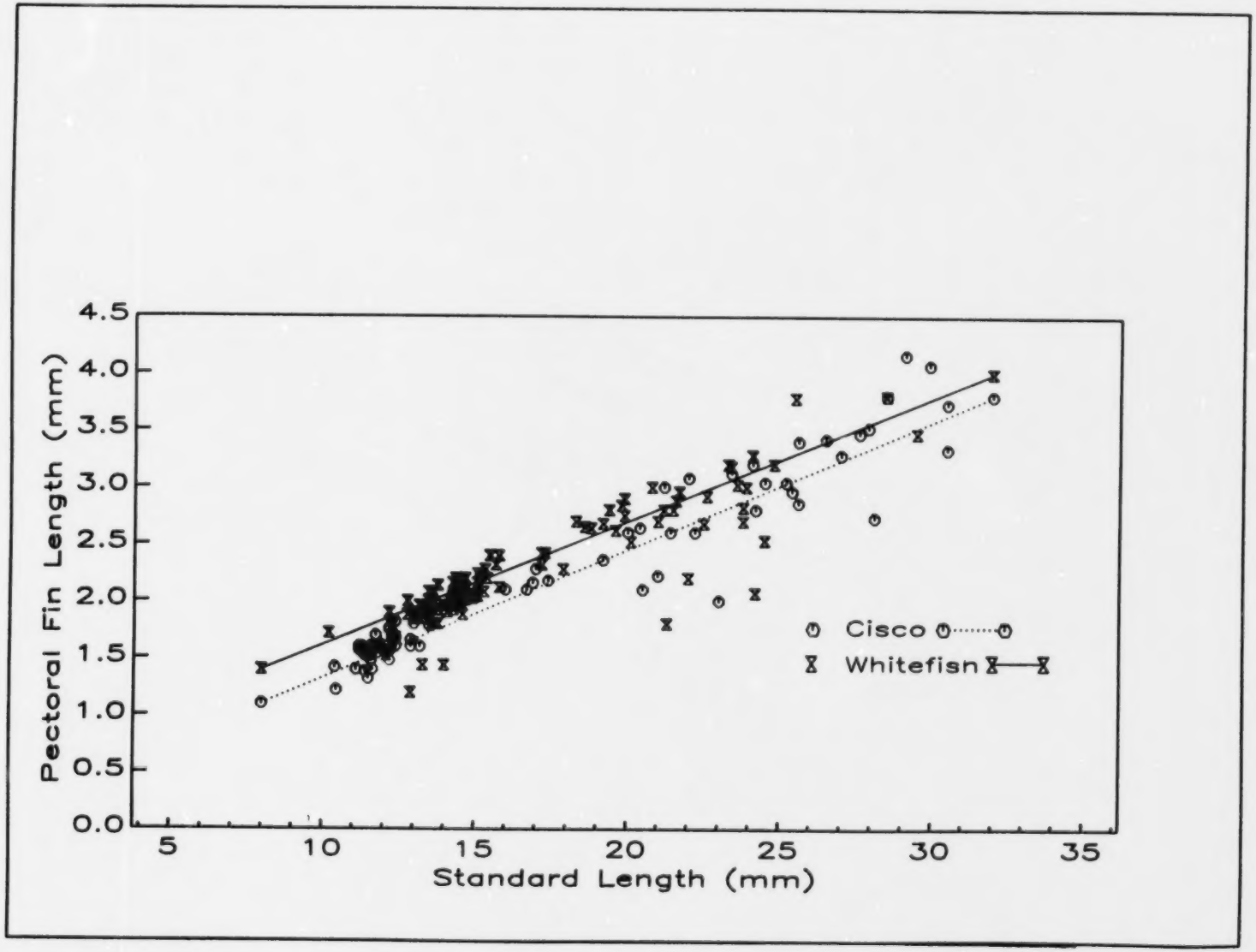

Fig. 9. Pectoral fin length versus standard length for hatchery reared lake whitefish and cisco.

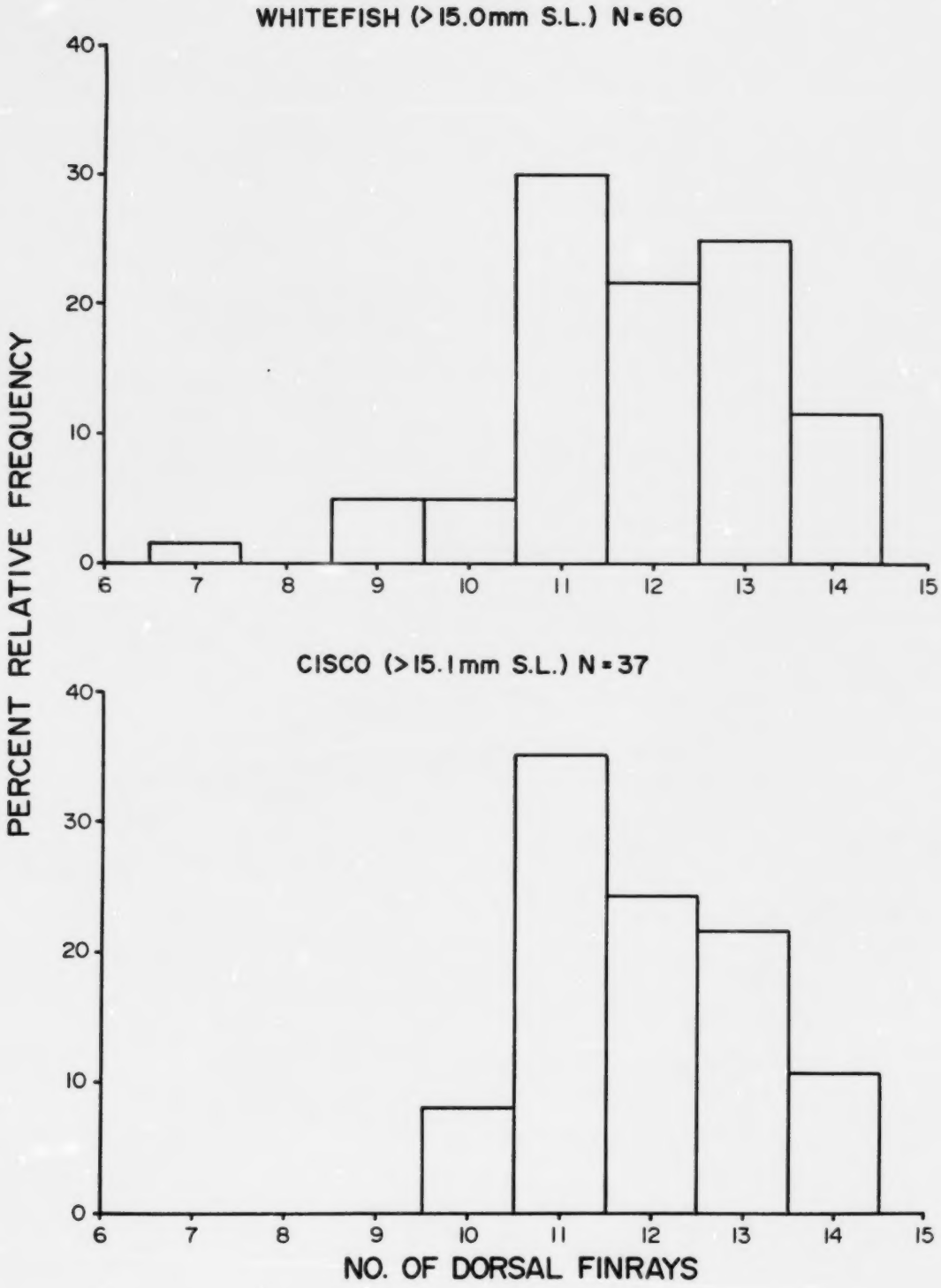

Fig. 10. Dorsal finray count frequency distributions for hatchery reared lake whitefish and cisco.

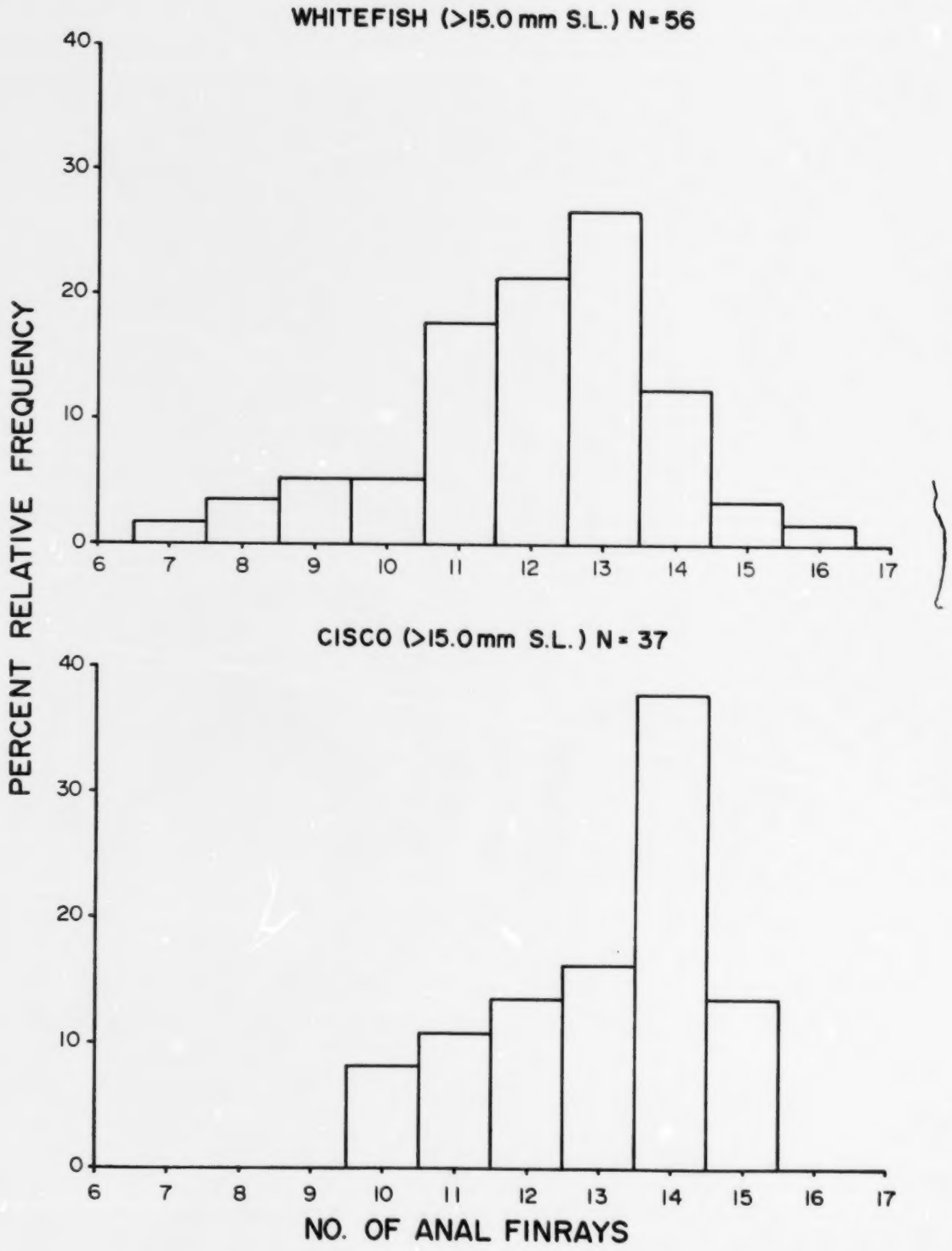

Fig. 11. Anal finray count frequency distributions for hatchery reared lake whitefish and cisco.

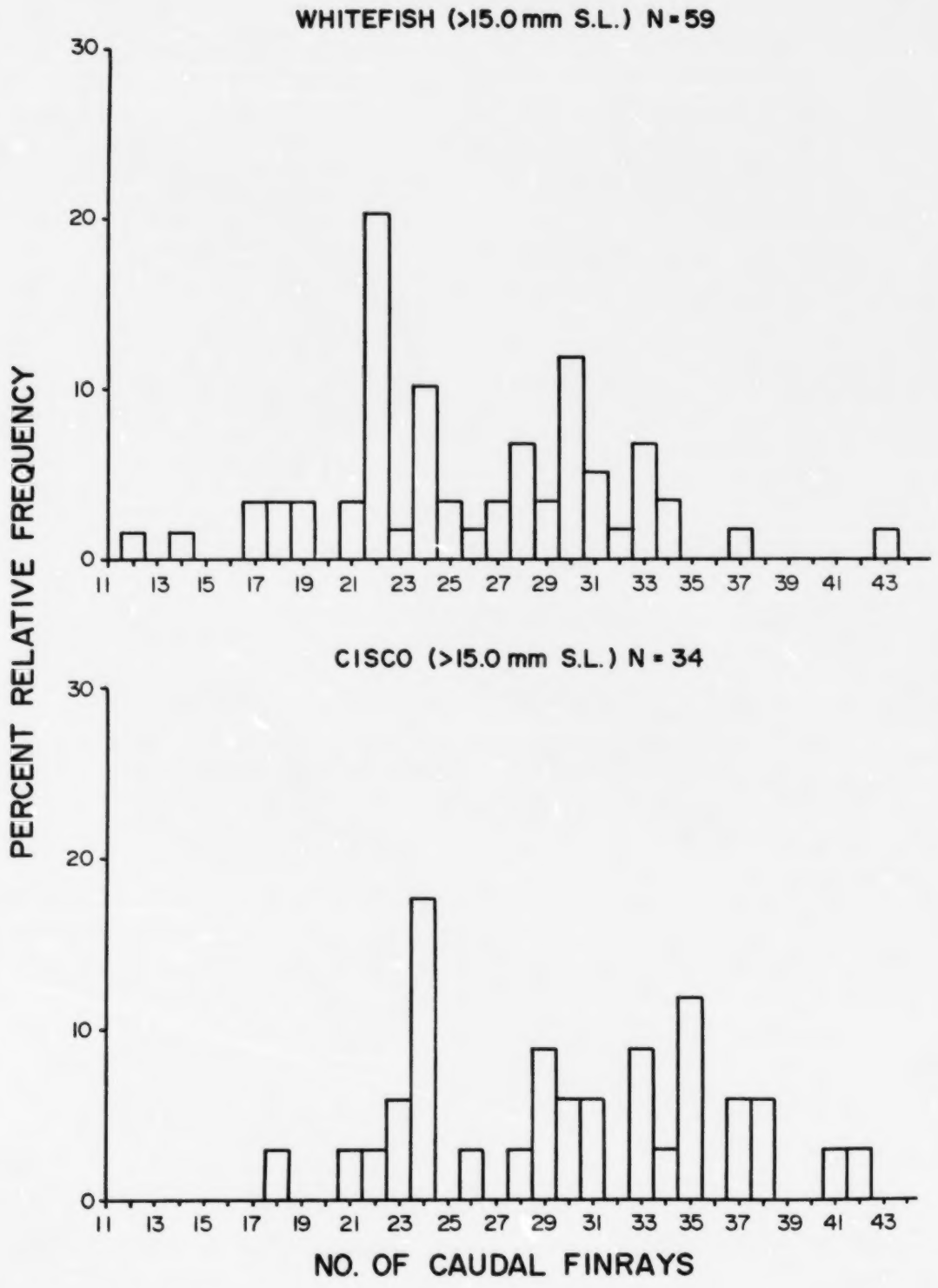
WHITEFISH (>15.0 mm S.L.) N = 59
CISCO (>15.0 mm S.L.) N = 34
PERCENT RELATIVE FREQUENCY
NO. OF CAUDAL FINRAYS
0
10
20
30
11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43

Fig. 12. Caudal finray count frequency distributions for hatchery reared lake whitefish and cisco.

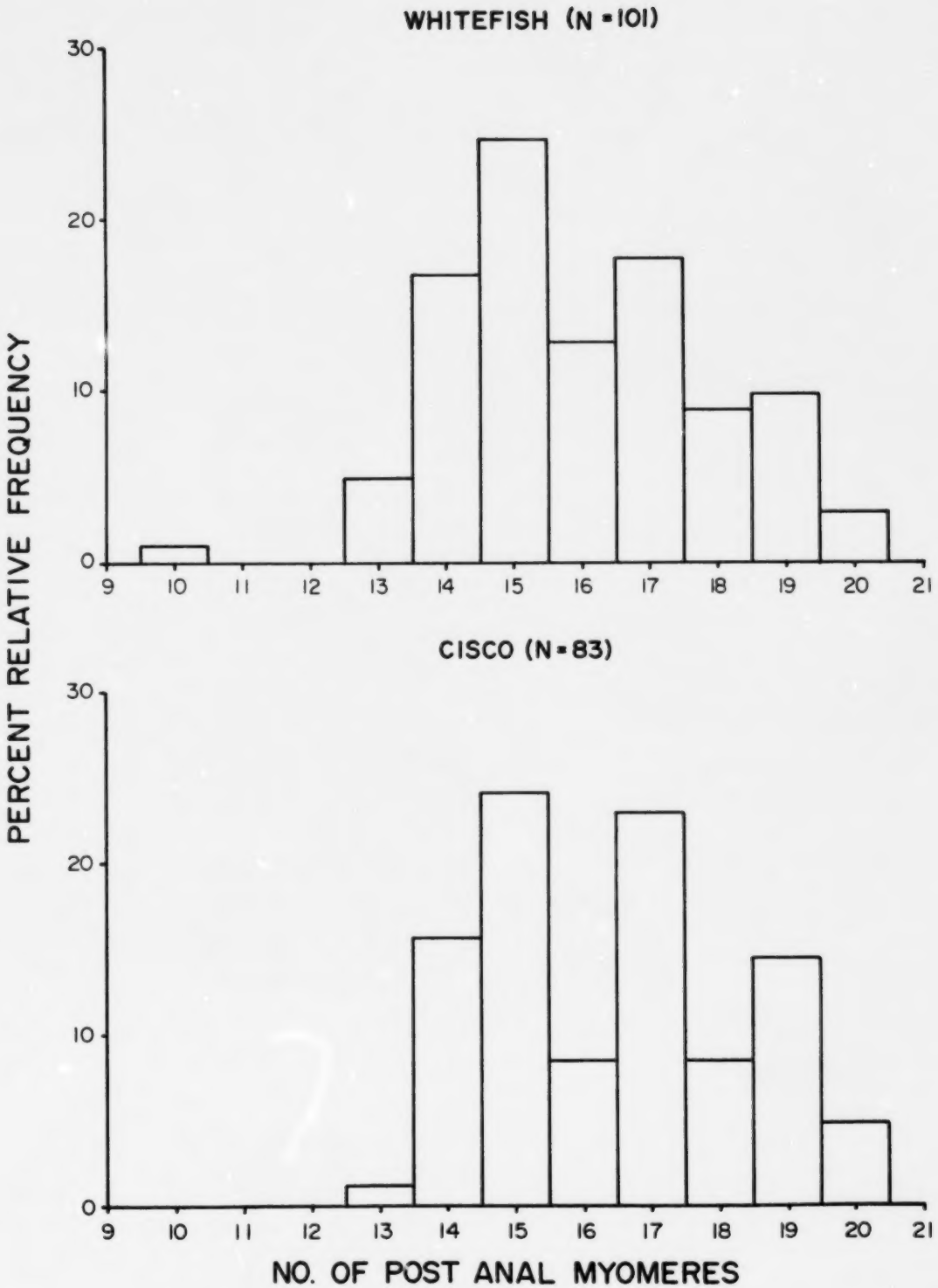

Fig. 13. Post-anal myomere count frequency distributions for hatchery reared lake whitefish and cisco.

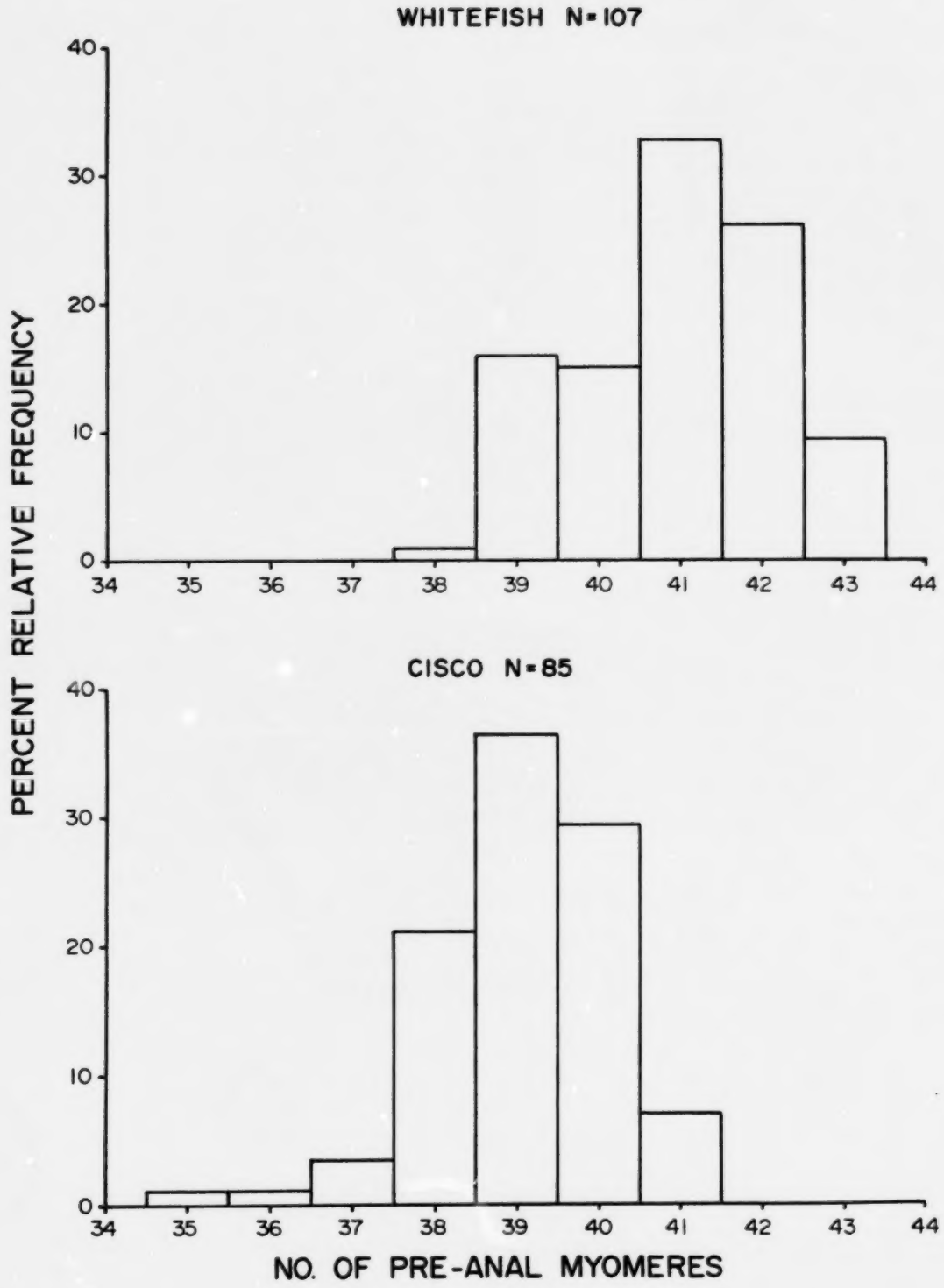

Fig. 14 Pre-anal myomere count frequency distributions for hatchery reared lake whitefish and cisco.

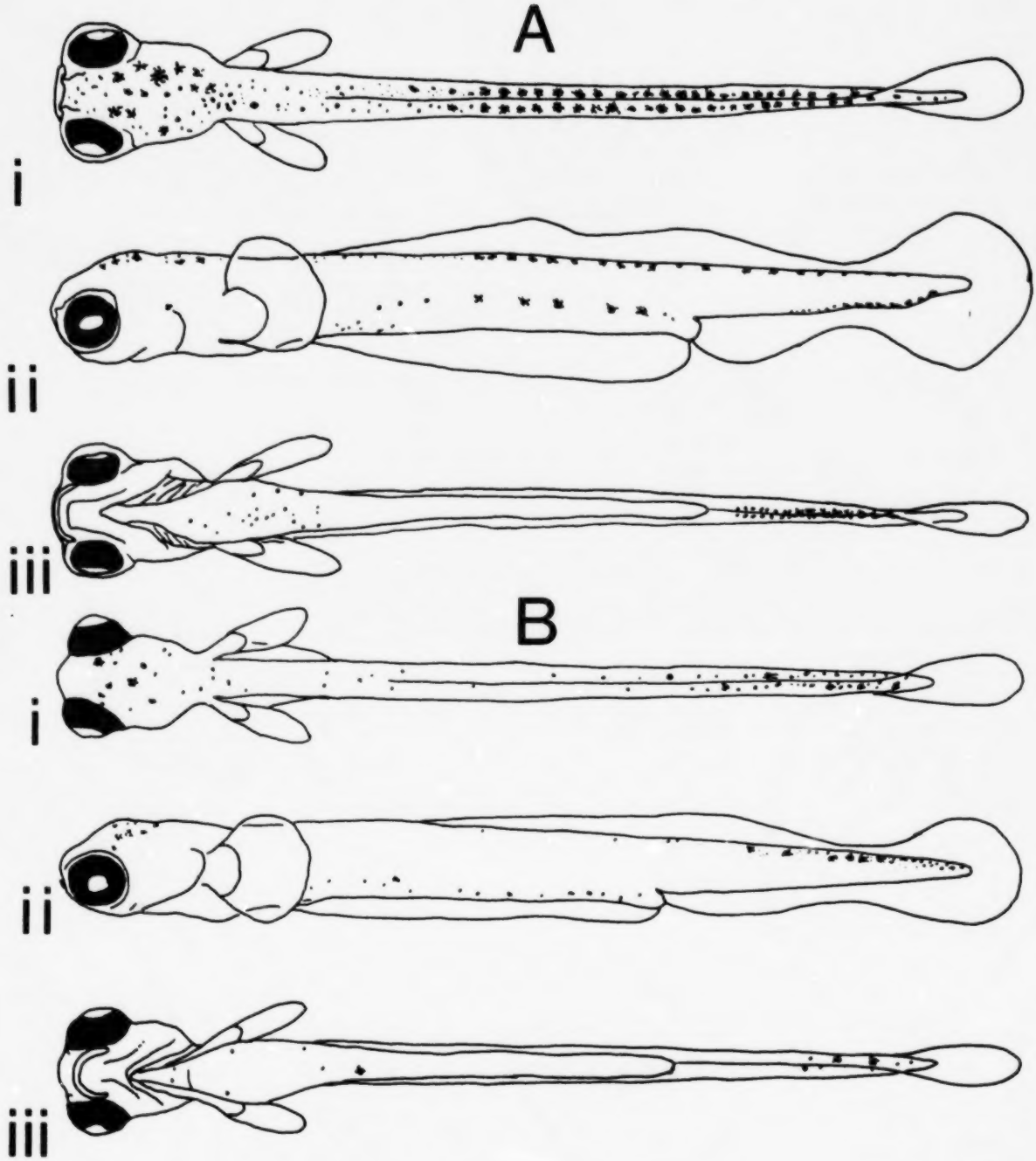

Fig. 15. (A) Larval lake whitefish (13.0 mm S.L.) - typical external pigmentation. (i) Dorsal (ii) Lateral (iii) Ventral.
(B) Larval cisco (13.0 mm S.L.) - typical external pigmentation.
(i) Dorsal (ii) Lateral (iii) Ventral.

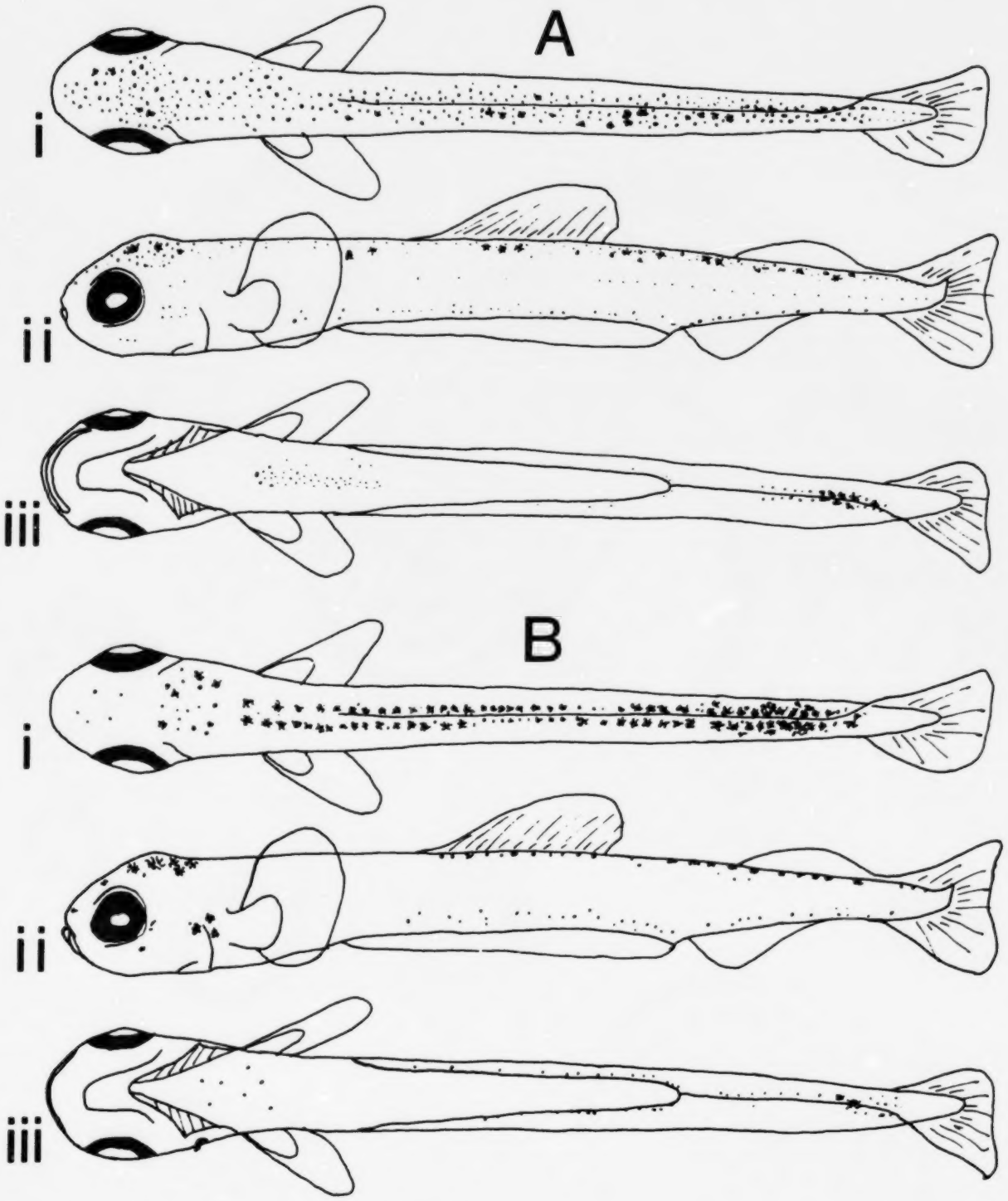

Fig. 16. (A) Larval lake whitefish (18.0 mm S.L.) - typical external pigmentation. (i) Dorsal (ii) Lateral (iii) Ventral.
(B) Larval cisco (18.0 mm S.L.) - typical external pigmentation. (i) Dorsal (ii) Lateral (iii) Ventral.

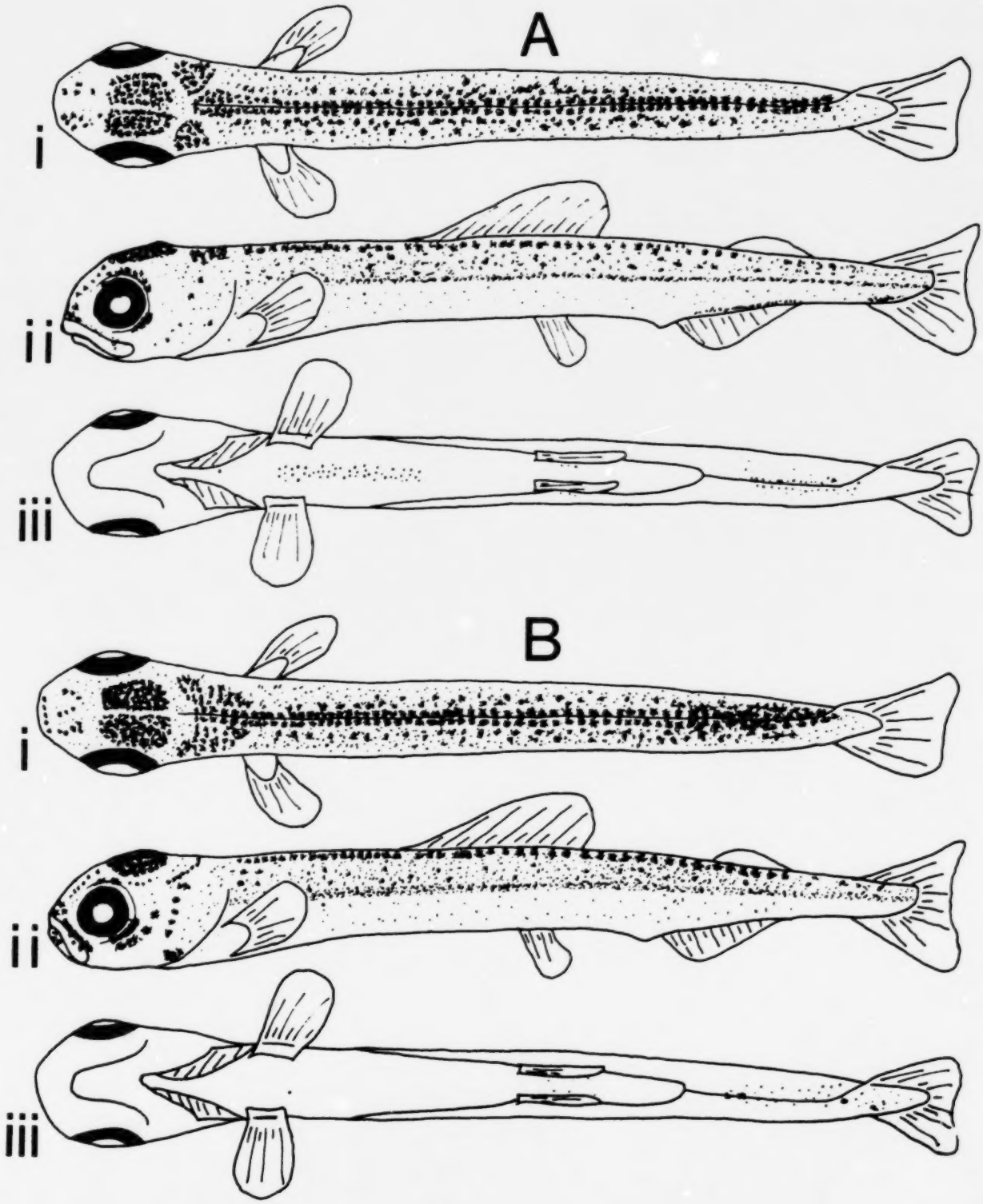

Fig. 17. (A) Larval lake whitefish (29.0 mm S.L.) - typical external pigmentation. (i) Dorsal (ii) Lateral (iii) Ventral.
(B) Larval cisco (29.0 mm S.L.) - typical external pigmentation.
(i) Dorsal (ii) Lateral (iii) Ventral.

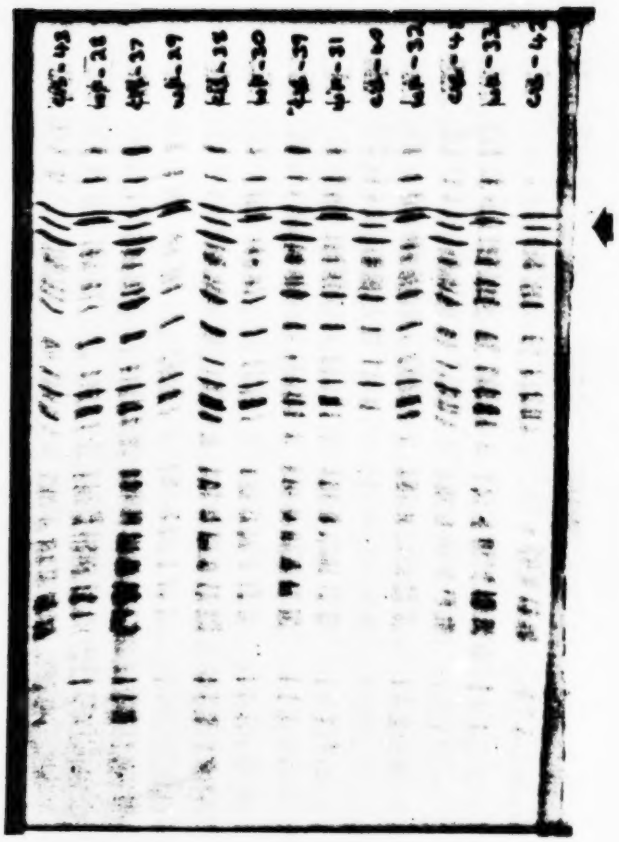

Fig. 18. Typical isoelectric focusing gel - showing species specific banding patterns for lake whitefish (WF) and cisco (CIS).